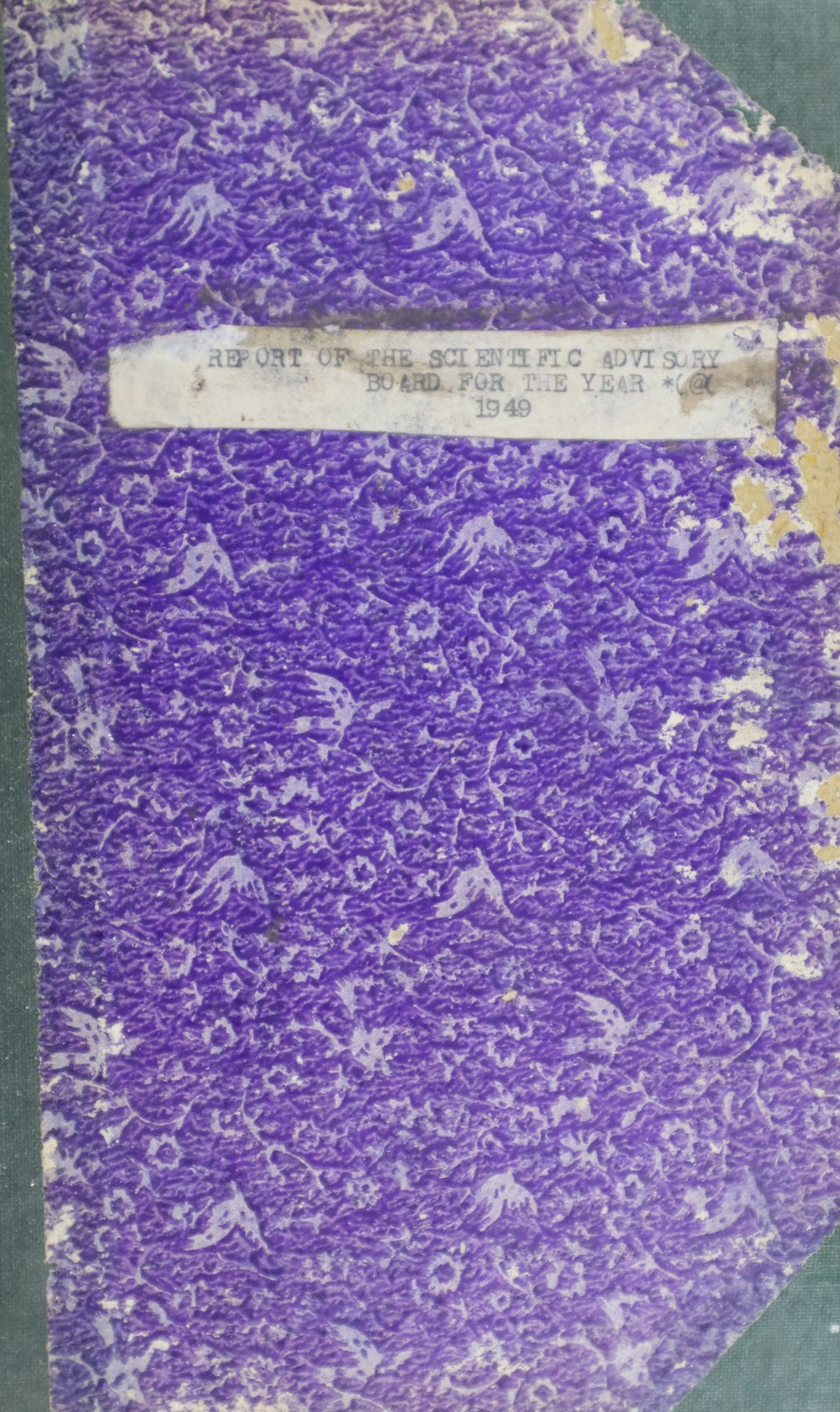


REPORT OF THE SCIENTIFIC ADVISORY
BOARD FOR THE YEAR *(@(
1949

CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE,
LIBRARY
23-6-50.

REPORT

OF THE

SCIENTIFIC ADVISORY BOARD

FOR THE YEAR 1949

Issued under the authority of the Governing Body,
Indian Research Fund Association,
New Delhi,
India.

Price per copy One Rupee.

Obtainable from the Secretary, Indian Research Fund Association,
Central Secretariat,
NEW DELHI (India).

Printed at the Job Press Ltd., Kanpur.

GOVERNING BODY

President.

The Hon'ble Rajkumari Amrit Kaur, Minister-in-Charge, Ministry of Health, Government of India, New Delhi.

Members.

P. M. Menon, Esqr., I.C.S., Joint Secretary to the Government of India, Ministry of Health, New Delhi.

Dr. K. C. K. E. Raja, L.M.S., L.R.C.P. & S., L.R.F.P.S., D.P.H., D.T.M. & H., Director-General of Health Services, New Delhi.

Lieut.-Colonel T. C. Puri, M.B.B.S., D.P.H., D.T.M. & H., Deputy Director-General of Health Services, New Delhi.

Lieut.-Colonel M. L. Ahuja, M.B., Ch.B., M.D., D.P.H., Director, Central Research Institute, Kasauli.

Lieut.-Colonel C. K. Lakshmanan, L.M. & S., M.R.C.P., D.T.M. & H., D.P.H., Director, All-India Institute of Hygiene & Public Health, Calcutta.

Dr. J. C. Gupta, M.B., D.T.M., F.N.I., Director, School of Tropical Medicine, Calcutta.

Maharaja Sir Sri Krishna Chandra Gajapathi Narayana Deo, Maharaja of Parlakimedi, District Ganjam.

Dr. J. N. Mukherji, D.Sc., F.C.S., F.R.A.S.B., F.N.I., Director, Indian Agricultural Research Institute, New Delhi.

Acharya J. B. Kripalani, 6, Jantar Mantar Road, New Delhi.

Shri Shankar Dattaraya Deo, 3, Electric Lane, New Delhi.

Dr. B. Pattabhi Sitaramayya, 19, Canning Lane, New Delhi.

The Hon'ble Dr. Jivraj N. Mehta, M.D., M.R.C.P., Minister for Public Works, Government of Bombay, Bombay.

Dr. Basudeva Narayana, M.Sc., M.B., Ph.D., F.R.S.E., F.N.I., Principal and Professor of Physiology, Department of Physiology, Prince of Wales Medical College, Patna.

Dr. S. S. Misra, M.D., M.R.C.P., Professor of Clinical Medicine, King George's Medical College, Lucknow.

Secretary.

Dr. C. G. Pandit, M.B.B.S., Ph.D., D.P.H., D.T.M., F.N.I., Secretary, Indian Research Fund Association, New Delhi.

CONTENTS

PAGE

I. COMPOSITION OF THE SCIENTIFIC ADVISORY BOARD vii
II. COMPOSITION OF THE ADVISORY COMMITTEES FOR THE YEAR 1950 ix
III. MEETINGS HELD BY THE SCIENTIFIC ADVISORY BOARD IN 1949 ... xii
IV. RESEARCHES CARRIED OUT DURING THE YEAR 1

CHOLERA.

1. Inquiry on cholera under the Director, Central Research Institute, Kasauli 1
2. Inquiry on cholera under Dr. K. V. Venkatraman, Director, King Institute, Guindy, Madras 5
3. Inquiry on cholera under the Director, School of Tropical Medicine, Calcutta 7

Report of the Cholera Advisory Committee 9

MALARIA.

1. Insecticide and mosquito repellent inquiry under the Director, Malaria Institute of India, Delhi 13
2. Inquiry on malaria under Dr. R. N. Chaudhuri at the School of Tropical Medicine, Calcutta 15
3. Inquiry on the chemotherapy of malaria under Dr. K. Ganapathi at the Haffkine Institute, Bombay 20
4. Inquiry on complement-fixation test in malaria for the assessment of anti-malarial drugs under Dr. J. C. Ray at the Indian Institute for Medical Research, Calcutta ... 21

Report of the Malaria Advisory Committee 24

NUTRITION.

1. Nutrition researches under Dr. V. N. Patwardhan at the Nutrition Research Laboratories, Coonoor 33
2. Nutrition research unit under Professor B. C. Guha at the University College of Science & Technology, Calcutta ... 48
3. Nutrition research unit under Dr. R. G. Chitre at the Seth G. S. Medical College, Bombay 56
4. Inquiry on the nutritive value of soya-bean milk and nutrition research unit under Mr. B. N. Banerjee and Dr. S. S. De at the Indian Institute of Science, Bangalore 60
5. Inquiry into metabolism of vitamin A under Dr. K. Rajagopal at the All-India Institute of Hygiene & Public Health, Calcutta 64
6. Inquiry on the preparation of anti-rachitic substances under Professor B. V. Bhide at the Maharaja Pratap Sinh Chemical Laboratory, S. P. College, Poona 67

PAGE

7. Inquiry into the effects of the malnutrition on the physique and more particularly on the blood picture including hæmoglobin and plasma-protein concentration as also the treatment thereof with high-protein diet, protein hydrolysates, etc., under Professor (Miss) S. Chaudhury and Dr. K. Mitra at the Lady Hardinge Medical College Hospital, New Delhi 67
8. Inquiry on the biosynthesis of nicotinic acid under Dr. S Banerjee at the Presidency College, Calcutta 69
9. Inquiry on the physiological effects of dietary protein under Dr. M. Damodaran at the National Chemical Laboratory, Poona 76

List of papers published on nutrition research 77

Report of the Nutrition Advisory Committee 78

LEPROSY.

1. Leprosy inquiry under Dr. Dharmendra at the School of Tropical Medicine, Calcutta 104
2. Inquiry on the histopathology of leprosy under Dr. R. G. Cochrane at the Government Lady Willingdon Leprosy Sanatorium, Chingleput 113

List of papers published on leprosy research 116

Report of the Leprosy Advisory Committee 117

PLAGUE.

1. Plague researches under the Director, Haffkine Institute, Bombay 124
2. Plague inquiry in the Nilgiris district under the Director of Public Health, Madras 126
3. Inquiry into the epidemiological factures of plague in Calcutta under Drs. R. B. Lal and S. C. Seal at the All-India Institute of Hygiene & Public Health, Calcutta 131

Report of the Plague Advisory Committee 140

CLINICAL RESEARCH.

1. Clinical research unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay 144
2. The neuropathological unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay 144
3. Inquiry on the role of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay 145
4. Clinical and biochemical investigations on certain aspects of infantile hepatic cirrhosis under Professor V. Subrahmanyan and Dr. V. R. Naidu at the Central Food Technological Research Institute, Mysore and Shri Krishnarajendra Hospital, Mysore 150

PAGE

5. Inquiry on typhus in Bengal under Dr. K. V. Krishnan at the All-India Institute of Hygiene & Public Health, Calcutta ... 161
6. Inquiry on the effects of vitamins B and C in diabetes under Dr. S. Banerjee at the Presidency College, Calcutta ... 162
7. Inquiry into erythroblastosis fœtalis and the Rh factor under Lieut.-Colonel S. D. S. Greval at the School of Tropical Medicine, Calcutta 165
8. Inquiry to investigate allergic diseases and significance of endogenous allergens as regards ætiology, diagnosis and treatment of allergic diseases under Dr. J. C. Ray at the Indian Institute for Medical Research, Calcutta 165
9. Inquiry on the immunological properties of lens and uveal proteins under Dr. S. N. Cooper at the Seth G. S. Medical College, Bombay 172
10. Inquiry on the neurogenic factor in the production of traumatic shock and its treatment by alteration of cerebro-spinal, fluid electrolytes under Dr. P. K. Sen at the Seth G. S. Medical College, Bombay 179
11. Investigation into the chromatophorotropic hormone of the pituitary gland and its employment in the treatment of pigmentary disorders of the skin under Dr. B. Mukerji at the Central Drugs Laboratory, Calcutta 183
12. Inquiry into the state of refraction of Indian children of different communities with special reference to myopia under Dr. G. S. Pendse at the Telegaon General Hospital, Poona ... 186
13. Study of *Monilia* infection of the skin and adjacent mucous membrane under Dr. L. M. Ghosh at the School of Tropical Medicine, Calcutta 187
14. Inquiry on the problems of typing typhoid bacilli by means of Vi-bacteriophage and treatment of typhoid fever with type-specific Vi-'phage under Drs. R. G. Dhayagude and D. D. Banker at the Seth G. S. Medical College, Bombay ... 189
15. Inquiry on circulatory changes in severe infections under Dr. R. N. Chaudhuri at the School of Tropical Medicine, Calcutta 192
16. Hæmatological unit under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta 196
17. Inquiry on the associated vaccine for prophylactic immunization against typhoid, paratyphoid, cholera, dysentery and tetanus by a single inoculation under Dr. J. C. Ray at the Indian Institute for Medical Research, Calcutta 198
18. Inquiry on the histological examination of the brain of *Microchiroptera taphozous melanopogen*. with special reference to auditory tracts and medial geniculate body under Dr. (Miss) L. Chacko at the Christian Medical College, Vellore ... 201

List of papers published on clinical research 202

Report of the Clinical Research Advisory Committee ... 204

PAGE

MATERNITY AND CHILD WELFARE.

1. Inquiry into the morbidity of children between the ages of 1 and 5 years (pre-school) in the Singur Health Centre area under Dr. Muktha Sen at the All-India Institute of Hygiene & Public Health, Calcutta 223
2. Inquiry into the vital losses of pregnancy and infancy in a rural population under Dr. Muktha Sen and Mr. K. K. Mathen at the All-India Institute of Hygiene & Public Health, Calcutta 224

Report of the Maternity and Child Welfare Advisory Committee 226

FILARIASIS.

1. Filariasis inquiry under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta 229
2. Inquiry to test the usefulness of intradermal and other immunological tests in the diagnosis of early filariasis under the Director, King Institute, Guindy, Madras ... 230
3. Inquiry on changing the physical properties of drugs by wetting agents under Dr. B. B. Dikshit, at the B. J. Medical College, Poona and Dr. K. Venkatraman of the Technology Department of the University of Bombay 234
4 Filaria control unit under the Director, Malaria Institute of India, Delhi 238

List of papers published on filariasis 240

Report of the Filariasis Advisory Committee 241

INDUSTRIAL HEALTH.

1. Industrial health research unit under the Director, All-India Institute of Hygiene & Public Health, Calcutta ... 244
2. Inquiry into the environmental conditions such as lighting, temperature, humidity, ventilation and dust in factories in and around Calcutta under Professor K. Subrahmanyan at the All-India Institute of Hygiene & Public Health, Calcutta ... 250
3. Inquiry into the problem of traffic accidents and accident-prone personnel amongst bus and tram drivers in Calcutta under Mr. S. K. Bose at the University College of Science & Technology, Calcutta 256
4. Inquiry into sickness absenteeism due to accidents under the Chief Medical Officer, Bata Shoe Factory, Batanagar 258
5. Inquiry into the prevalence of byssinosis in cotton textile industry in South India under Dr. T. S. Adisubrahmaniam, Municipal Health Officer, Mathurai 259

Report of the Industrial Health Advisory Committee ... 266

RABIES.

Report of the Rabies Advisory Committee 271

PHARMACOLOGY.

PAGE

1. Inquiry on indigenous drugs under Dr. J. C. Gupta at the School of Tropical Medicine, Calcutta 276
2. Inquiry on indigenous drugs under Brevet-Colonel R. N. Chopra at the Drug Research Laboratory, Jammu ... 279
3. Inquiry on the action of snake venoms and their constituents on the biochemical activities of tissue-cells under Dr. B. N. Ghosh at the University College of Science & Technology, Calcutta 281

Report of the Pharmacology Advisory Committee ... 285

MISCELLANEOUS RESEARCHES.

1. Inquiry on filterable viruses under the Director, King Institute, Guindy, Madras 291

V. *Indian Journal of Medical Research* AND *Indian Medical Research Memoirs* 294

VI. *Indian Journal of Malariology* 297

VII. PAPERS PUBLISHED ON MEDICAL RESEARCH CARRIED OUT UNDER THE AUSPICES OF THE INDIAN RESEARCH FUND ASSOCIATION DURING THE YEAR 1949 298

VIII. PROGRAMME OF RESEARCHES AND MISCELLANEOUS GRANTS RECOMMENDED FOR THE YEAR 1950-51 302

I. COMPOSITION OF THE SCIENTIFIC ADVISORY BOARD.

The Scientific Advisory Board consisted of the following :—

Chairman.

Dr. K. C. K. E. Raja, L.M.S., L.R.C.P. & S., L.R.F.P.S., D.P.H., D.T.M. & H., Director-General of Health Services, New Delhi.

Members.

Major-General A. C. Chatterji, M.B., D.P.H., Dr. P.H., Director of Health Services, West Bengal, Calcutta.

Dr. B. B. Dikshit, M.D., M.R.C.P., Ph.D., D.P.H., F.N.I., Principal, B. J. Medical College, Poona.

Lieut.-Colonel Jaswant Singh, M.B., Ch.B., D.T.M. & H., D.P.H., Director, Malaria Institute of India, Delhi.

Dr. V. R. Khanolkar, B.Sc., M.D., Director of Laboratories & Research, Tata Memorial Hospital, Bombay.

Lieut.-Colonel C. K. Lakshmanan, L.M. & S., M.R.C.S., D.T.M. & H., D.P.H., Director, All-India Institute of Hygiene & Public Health, Calcutta.

Dr. B. Mukerji, D.Sc., M.D., F.N.I., Director, Central Drugs Laboratory, 110, Chittaranjan Avenue, Calcutta.

Colonel S. Narain, I.A.M.S., Deputy Director (Hygiene), Medical Directorate, D.M.S.(5), Army Headquarters, New Delhi.

Lieut.-Colonel B. S. Nat, M.D., Ch.B., F.R.C.S., Inspector-General of Civil Hospitals, East Punjab, Simla.

Dr. (Miss) S. Pandit, W.M.S., Director, Maternity & Child Welfare Bureau, Indian Red Cross Society, & Adviser on Maternity and Child Welfare, Directorate-General of Health Services, New Delhi.

Dr. D. N. Roy, M.D., D.Sc., D.T.M., 53-B, Southern Avenue, Calcutta.

Major-General S. S. Sokhey, B.Sc., M.A., M.D., Ch.B., D.T.M. & H., F.A.S., F.N.I., Director, Haffkine Institute, Bombay.

Dr. G. Sankaran, M.B.B.S., Professor of Biochemistry & Nutrition, All-India Institute of Hygiene & Public Health, Calcutta.

Dr. K. V. Venkatraman, M.D., Director, King Institute, Guindy, Madras.

Dr. B. B. Yodh, M.B., M.R.C.P., D.T.M. & H., Rawal Building, Lamington Road, Bombay.

Co-opted member.

The Hon'ble Dr. Jivraj N. Mehta, M.D., M.R.C.P., Minister of Public Works, Government of Bombay, Bombay.

Secretary.

Dr. C. G. Pandit, M.B.B.S., D.P.H., D.T.M., Ph.D., F.N.I., Secretary, Indian Research Fund Association, New Delhi.

II. COMPOSITION OF THE ADVISORY COMMITTEES FOR THE YEAR 1950.

I. Cholera.

1. Major-General S. S. Sokhey. (*Chairman*).
2. Lieut.-Colonel M. L. Ahuja.
3. Major-General A. C. Chatterji.
4. Dr. C. Chandra Sekar.
5. Dr. K. V. Krishnan.
6. Dr. R. B. Lal.
7. Dr. C. G. Pandit.
8. Dr. S. R. Pandit.
9. Dr. K. V. Venkatraman. (*Secretary*).

II. Malaria.

1. Lieut.-Colonel Jaswant Singh. (*Chairman*).
2. Dr. A. C. Banerjea.
3. Brevet-Colonel R. N. Chopra.
4. Dr. A. K. Ganapathi.
5. Dr. M. O. T. Iyengar.
6. Dr. B. Ananthaswamy Rao.
7. Dr. D. N. Roy.
8. Dr. D. K. Viswanathan.
9. Dr. K. S. Viswanathan.
10. Dr. I. M. Puri. (*Secretary*).

III. Nutrition.

1. Dr. G. Sankaran. (*Chairman*).
2. Dr. S. Banerji.
3. Dr. K. P. Basu.
4. Dr. M. Damodaran.
5. Dr. B. B. Dikshit.
6. Dr. B. C. Guha.
7. Dr. K. Mitra.
8. Dr. M. V. Radhakrishna Rao.
9. Dr. V. Shubrahmanyan.
10. Dr. V. N. Patwardhan. (*Secretary*).

IV. Plague.

1. Major-General S. S. Sokhey. (*Chairman*).
2. Dr. A. M. Francis.
3. Dr. R. B. Lal.
4. Dr. Dev Raj Mehta.
5. Dr. C. G. Pandit.
6. Dr. K. V. Venkatraman.
7. Dr. P. M. Wagle. (*Secretary*).

V. Clinical Research.

1. Dr. V. R. Khanolkar. (*Chairman*).
2. Dr. R. N. Chaudhuri.
3. Dr. R. N. Cooper.
4. Dr. C. R. Das Gupta.
5. Dr. M. G. Kini.
6. Dr. P. Kutumbiah.
7. Dr. B. Mukerji.
8. Colonel S. Narain.
9. Dr. B. B. Yodh.
10. Dr. J. C. Patel. (*Secretary*).

VI. Rabies.

1. Lieut.-Colonel M. L. Ahuja. (*Chairman*).
2. Dr. K. Banerjee.
3. Dr. C. B. D'Silva.
4. Lieut.-Colonel S. D. S. Greval.
5. Dr. S. R. Pandit.
5. Dr. R. Sanjiva Rao.
7. Dr. N. Veeraraghavan.
8. Dr. K. V. Venkatraman.
9. Dr. A. G. Brooks. (*Secretary*).

VII. Leprosy.

1. Dr. C. G. Pandit. (*Chairman*).
2. Dr. S. N. Chatterjee.
3. Dr. R. G. Cochrane.
4. Dr. N. Figueredo.
5. Lieut.-Colonel B. N. Harjra.
6. Dr. V. R. Khanolkar.
7. Dr. Dharmendra. (*Secretary*).

VIII. Filariasis.

1. Dr. C. G. Pandit. (*Chairman*).
2. Dr. R. G. Dhayagude.
3. Dr. B. B. Dikshit.
4. Dr. Sangham Lal.
5. Dr. A. K. Mukerjee.
6. Dr. C. K. Prasada Rao.
7. Dr. D. N. Roy.
8. Lieut.-Colonel Jaswant Singh.
9. Dr. M. O. T. Iyengar. (*Secretary*).

IX. Industrial Health.

1.	Lieut.-Colonel C. K. Lakshmanan.	(*Chairman*).
2.	Dr. T. S. Adisubramaniam.	
3.	Major V. M. Albuquerque.	
4.	Dr. A. C. Banerjea.	
5.	Mr. M. S. Mankiker.	
6.	Dr. (Miss) S. Pandit.	
7.	Dr. K. Rajagopal.	
8.	Dr. L. M. Sen.	
9.	Professor K. Subrahmanyan.	
10.	Dr. M. N. Rao.	(*Secretary*).

X. Maternity and Child Welfare.

1.	Dr. J. Jhirad.	(*Chairman*).
2.	Dr. S. T. Achar.	
3.	Dr. A. Benjamin.	
4.	Dr. Coelho.	
5.	Dr. B. C. Das Gupta.	
6.	Dr. C. R. Das Gupta.	
7.	Dr. Muktha Sen.	
8.	Dr. D. M. Satur.	
9.	Dr. Satya Swaroop.	
10.	Dr. (Miss) S. Pandit.	(*Secretary*).

XI. Pharmacology.

1.	Brevet-Colonel R. N. Chopra.	(*Chairman*).
2.	Dr. B. Mukerji.	
3.	Dr. B. B. Dikshit.	
4.	Dr. B. N. Ghosh.	
5.	Dr. J. C. Gupta.	
6.	Dr. B. B. Yodh.	
7.	Mr. P. M. Nabar.	(*Secretary*).

III. MEETINGS HELD BY THE SCIENTIFIC ADVISORY BOARD IN 1949.

The Board held its annual meeting on the 28th and 29th November, 1949 and considered in detail the programme of research for the year 1950-51 and recommended the programme given on pages 302 to 307 of this Report.

Technical Report of the Scientific Advisory Board of the Indian Research Fund Association for the year 1949.

RESEARCHES CARRIED OUT DURING THE YEAR.

The researches which were carried out during the year under report were recommended by the Board at its meeting held in New Delhi on the 3rd and 4th December, 1948, and were approved by the Governing Body of the Indian Research Fund Association at its meeting held in New Delhi on the 19th March, 1949.

CHOLERA.

1. Inquiry on cholera under the Director, Central Research Institute, Kasauli.

The purpose of this inquiry is to study questions relating to the immunizing efficiency of vibrio polysaccharides and other allied problems having a bearing on the antigenic constitution of *V. choleræ.*

The following is a brief account of the work of the inquiry during the year 1949-50 :—

Immuno-chemical studies.—In our report presented to the Cholera Advisory Committee of the Indian Research Fund Association in December 1948 it has been shown that a polysaccharide fraction isolated from *V. choleræ* Inaba was able to confer immunity against cholera infection in experimental animals. This line of work had been continued, with polysaccharide fractions of both Inaba and Ogawa subtypes of vibrios.

Technique for fractionation of polysaccharide was the same as previously reported, i.e. phenol treatment of acetone-dried growth of *V. choleræ* and subsequent precipitation of the polysaccharide with 95 per cent alcohol.

Mice 15 g. to 20 g. in weight were used as test animals. They were given two doses sub-cutaneously of 0·2 c.c. each of the polysaccharide solution under test at weekly intervals, followed a week later by an intraperitoneal injection of a mucinized suspension of *V. choleræ.*

Polysaccharide fractions of nine strains, five of Inaba and four of Ogawa were tested for their protective efficiency. Details of the immunizing efficiency, precipitin titre and nitrogen content of the fractions tested are shown in Table I.

At the same time another fraction—Inaba 194A—which had previously been found to confer a high degree of immunity was also tested for its minimum protecting dose. Storage of this fraction in a dry atmosphere over P_2O_5 for a period of ten months resulted in marked deterioration of its protective efficiency. The results of these tests are recorded in Table II.

The results confirm our previous finding that it is possible to isolate from both Inaba and Ogawa strains polysaccharide fractions of high antigenicity.

It was noticed that a polysaccharide fraction reacting in high titre to cholera precipitin sera does not necessarily confer a high degree of immunity. Work is in progress on testing the immunizing value of freshly prepared vibrio polysaccharides in human volunteers.

Rough and smooth strains.—An attempt was made to transform a fully rough vibrio into a smooth strain. A rough varient of *V. choleræ* Inaba was grown in peptone water to which 10 per cent of polysaccharide isolated from a smooth strain of Inaba had been added. This polysaccharide fraction reacted to one in a million with cholera precipitin serum. Transfers were carried out daily for 20 passages and 24 hours growth was plated on nutrient agar. The least rough-looking colonies were tested against smooth '0' sera. No serological change was observed in any of the hundreds of colonies so tested and after the 20 passages there was no evidence of any reversion from a rough variant to a smooth type.

Parallel experiments were carried out using heat-killed suspensions of smooth Inaba vibrios which had previously been subjected to repeated freezing and thawing. This suspension was added to the nutrient medium in place of the vibrio polysaccharide. No change was observed either in colony appearance or in serological reaction of the strain under test. Work on partially rough strains is in progress, but so far as the fully rough variants are concerned, transformation to smooth types has not been obtained by the methods employed above.

It has, however, been noticed that as a result of frequent sub-passages on nutrient agar, the rough vibrio cultures have a progressive tendency towards reversion to a smoother state. This has been seen in the case of some of the fully rough strains maintained at this Institute. It must, however, be mentioned that these strains were derived originally from smooth types by the use of anti-serum treatment. A gradual dissociative change in their serology has been noticed. Some of the strains throw out colonies which react to as much as 25 to 50 per cent of the titre of either Ogawa or Inaba type-specific sera. By careful colony selection, however, fully rough variants can be maintained. It would be interesting to see the effect of prolonged storage of rough vibrios isolated from cases and convalescents.

Anaphylaxis.—An investigation was undertaken to see if the polysaccharide of *V. choleræ* could produce anaphylaxis in passively immunized animals.

Two groups of guinea-pigs were injected intraperitoneally with (*i*) Inaba type-specific serum and (*ii*) cholera '0' serum (non-differential) raised in rabbits. Twenty-four hours later both the groups were given intravenously a sublethal dose of polysaccharide isolated from a smooth strain of vibrio Inaba. None of the animals died or showed any visible signs of anaphylaxis.

Monospecific sera for typing of cholera strains.—In accordance with the recommendation of the Expert Group of the World Health Organization on cholera, preparation of monospecific cholera sera was undertaken at this Institute. Four hundred c.c. of type-specific sera—types of Inaba and Ogawa—have been prepared and will be sent to Copenhagen to be stocked as standard reference preparations for the typing of cholera strains.

A considerable amount of work requires to be done on the immuno-chemical properties of vibrio polysaccharides and it is proposed to continue the work during 1950-51, with special emphasis on the following:—

> Testing the immunizing efficiency of polysaccharide fraction of *V. choleræ* Inaba and Ogawa on human volunteers. Isolation of similar polysaccharide fractions from water vibrios to see whether the same immunizing property is present in vibrios from non-cholera sources. Testing the reversion effect of vibrio polysaccharides on partially rough vibrios. The phenol method of isolation of vibrio polysaccharides has not given consistent results in our hands, the product obtained from the strain showing different protective values in different batches. It is proposed to study and eliminate, if possible, from the method employed factors responsible for loss due to denaturing.

Table I

Active immunization of mice with polysaccharide fractions of V. choleræ.

Challenge dose.	Number of vibrios.	OGAWA								INABA										Cholera vaccine c.c.		Controls unvaccinated.
		PI		PII		PIII		PVIII		PIV		PV		PVI		PVII		PIX				
		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	
10—2	18,470,000	3/4	0/4	1/4	0/4	1/4	1/4	2/4	1/4	3/4	3/4	2/4	1/4	...	...	3/4	3/4	2/4	3/4	4/4	3/4	0/16
10—3	1,847,000	3/4	2/4	3/4	1/4	2/4	1/3	2/4	4/4	2/3	3/4	3/4	1/4	...	2/5	3/4	3/4	4/4	4/4	4/4	4/4	6/17
10—4	184,700																					11/17
10—5	18,470																					13/17
10—6	1,847																					17/20

Polysaccharide.			Total immunizing dose, mg.		Nitrogen content 0/0.	Precipitin titre.	
			Series A.	Series B.		Inaba.	Ogawa.
PI	Ogawa	62/I	0·004	0·0008	15·6	2,000	2,000,000
PII	Ogawa	292/I	0·0039	0·00078	6·1	20,000	200,000
PIII	Ogawa	292/II	0·0034	0·00068	6·4	200,000	2,000,000
PVIII	Ogawa	481445	0·00384	0·000768	7·6	200,000	200,000
PIV	Inaba	481770/I	0·004	0·0008	5·9	200,000	200,000
PV	Inaba	194/A/I	0·0028	0·00056	7·7	*2,000	Neg. in 2,000
PVI	Inaba	194/A/II	0·004	0·0008	5·7	200,000	20,000
PVII	Inaba	194/A/III	0·004	0·0008	6·9	2,000,000	20,000
PIX	Inaba	481771	0·00384	0·000768	8·2	200,000	*20,000

Numerators=Number survived. Denominators=Total number of animals. * Opacity but no deposit.

Cholera vaccine diluted 1/10 and 1/100 in series A and B respectively; immunizing dose=two doses of 0·2 c.c. each at week's interval.

TABLE II

Minimum protective dose of polysaccharide of V. choleræ 194/A/I *freshly isolated and after storage for 10 months.*
Date of test 19-8-48.

Challenge dose.	Number of vibrios.	Polysaccharide 194/A/I : Total amount of polysaccharide injected, mg.					Controls unvaccinated.	
		0·06	0·012	0·0024	0·00048	0·000088		
10^{-3}	1,935,000	4/5	4/5	7/10	4/4	4/5	1/10	Nitrogen content : 7·7 per cent Precipitin titre : Inaba—200,000 Ogawa—200,000
10^{-4}	193,500	4/4	5/5	7/9	3/5	3/5	2/10	
10^{-5}	19,350	4/4	5/5	8/9	4/5	4/5	4/10	
10^{-6}	1,935	...	...	...	...	...	7/10	
10^{-7}	195	...	...	...	...	...	8/10	
10^{-8}	23	...	...	...	...	...	10/10	
Date of test 11-6 49.				0·0028	0·00056			Precipitin titre : Inaba—*2,000 Ogawa—neg. 2,000
10^{-2}	17,750,000	...	...	2/4	1/4	...	0/4	
10^{-3}	1,775,000	...	...	3/4	1/4	...	1/4	
10^{-4}	177,500	...	...	...	...	...	2/4	
10^{-5}	17,750	...	...	...	...	...	4/4	
10^{-6}	1,775	...	...	...	...	...	4/4	

Numerators=Number survived. Denominators=Total number of animals.

* Opacity but no deposit.

2. Inquiry on cholera under Dr. K. V. Venkatraman, Director, King Institute, Guindy, Madras.

The scope of the inquiry was changed in accordance with the decision of the Co-ordinating Committee, to an investigation of the possibility of sub-clinical cases constituting a link between two cholera outbreaks. For this purpose it was considered essential that the field of observation should be one where successive outbreaks of cholera ordinarily occur almost regularly but not with such frequency as in known endemic areas where the maintenance of infection can, with reasonable probability, be attributed to a continuous series of apparent cases of cholera. It is also essential that the selected field should be sufficiently removed from known endemic areas to limit the possibility of frequent introductions of infection from such areas. Certain regions in the Cauvery Delta of the Tiruchirapalli and Tanjore districts in South India where annual epidemic outbreaks of cholera are common constitute an ideal location. It would have been desirable to include the whole of the Cauvery Delta or at least of the rural area included in a small compact belt but the non-availability of adequate funds and of a sufficient number of trained technical personnel for the purpose are limiting factors in the extent of the area selected for study. As a practical compromise a group of 30 villages together with their hamlets and *cheries* has been finally selected for close and continuous observation during the course of the inquiry. Before making this selection the recorded incidence of cholera during the past 10 years of all the villages included in the *taluks* of Tanjore district comprising the deltaic area and the Chidambaram taluk of the South Arcot district and the Lalgudi taluk of the Tiruchirapalli district were subjected to a close scrutiny. A small triangular belt comprising parts of Lalgudi taluk, the northern part of the Tanjore taluk and a portion of the Papanasam taluk was finally fixed on as being most suitable. The most frequent occurrence during the last 10-year period was a principal consideration in the selection of the individual villages but villages very near large populous centres attracting a heavy pilgrim traffic were excluded as it was considered that it would be very difficult in practice to exclude external sources of introduction of infection. For the same purpose, large towns and comparatively large *panchayats* with large populations were also excluded. The final list is comprised of four clusters of villages along the course of the Cauvery and its tributaries, some of which at least it is believed would be involved in any large outbreak of cholera in the delta.

The available recorded information of the population of these villages, namely the census figures of 1941 are not indicative of the present condition. A detailed survey of these villages has been undertaken and is in progress; the disposition of the streets and houses in relation to available sources of water-supply has been recorded. A fresh enumeration has been undertaken of the population and relevant details regarding age, sex, religion, occupation, health history and the inoculation of the members of each family residing in this area are under compilation. It is proposed that the health surveillance of each family will be based on actual visits and inquiries made at intervals of approximately a week by the Inspectors assigned to the villages. Specimens of stools of all individuals whose co-operation could be elicited are proposed to be obtained at a frequency of approximately 2 to 3 a month and submitted for examination for the detection of *V. choleræ* in the Field Laboratory located at Tiruchirapalli, the facilities of which are adequate for a daily examination of about 3,000 specimens. Certain sources of drinking water in and near the villages selected for observation are also to be subjected to repeated examinations for the isolation of *V. choleræ*.

In the collection of specimens, ½ oz. screw-capped bottles containing 5 c.c. of boric buffer saline labelled with the name and other particulars adequate to identify the specimen are handed out personally by the Inspector to a certain number of people in the village with instructions to collect a specimen of stool the next morning on a small swab and emulsify it in the medium and return these bottles containing specimens to the Inspector or his assistant after collection. These bottles are then forwarded to the Field Laboratory at Tiruchirapalli. With a view to expeditiously handling large numbers of stool specimens, they are examined in groups of ten, the contents of 10 bottles being included in a flask containing the required amount of a modified Wilson and Blair fluid enrichment medium and after 24 hours incubation plated out on a modified Reiley's solid medium. Adequate care is taken in pouring the contents to avoid infection of the original bottles which are set aside until the results of the pool are known. In the event of the isolation of *V. choleræ* from any pool, the small remnant of the specimen contained in each of the bottles constituting the pool will be similarly enriched and plated to determine which of these were contributory to the positive isolation from the pool. The practicability of this procedure has been demonstrated experimentally and during the course of a recent investigation of the staff of a city hospital where there occurred a small outbreak of cholera. In special cases, however, either because of the history of the patient or of a previous isolation, examination will be conducted on individual specimens.

The survey and enumeration of 16 villages have been completed and are nearing completion in the remaining 14 villages. Specimens of stools have just begun to come in and so far 2,479 specimens have been examined and all of them have been negative. The region comprising the selected villages has been free from cholera since the commencement of the investigation up to the present time.

As part of an investigation on the occurrence of cases of cholera in one of the hospitals in the city, arrangements were made to have the entire staff of the hospital examined with a view to ascertaining if any of them happened to be healthy excretors of *V. choleræ*. The opportunity was availed to make a modest scale try out of the technique that is proposed to be followed in the Field Laboratory at Tiruchirapalli. Stool specimens were collected daily under the supervision of the Resident Medical Officer of the hospital in boric buffer saline and transmitted to the Institute. With a view to getting through the larger number of examinations speedily and to save time, labour and material, collective examinations were made of groups of ten, except in the case of food handlers such as cooks, etc., whose specimens were actually examined individually. These specimens were enriched in a modified Wilson and Blair medium and plated on modified Reiley's solid medium. In this manner, a total of 2,240 specimens of stools obtained from 245 members of the staff including nurses, cooks, sweepers, peons, etc., were examined between the 9th and 29th August, 1949. Further examinations were discontinued in view of the fact that subsequent to the 16th of August, 1949, no isolation of *V. choleræ* was made from any of the persons examined. While it was intended that daily specimens should be obtained from each member of the staff, no specimens were received from 14, and less than three specimens were received from 18 others. In over 204 persons, seven or more specimens were obtained and examined. *V. choleræ* was isolated during these examinations on five occasions. In all these, the vibrio was isolated on only one occasion from each individual and subsequent daily examinations were negative. In all cases but one, where re-examination of the remnant contained in the individual sample bottles was undertaken, it was possible to make an isolation from those included in the group, and the failure to identify the incriminating specimen in the exceptional pool was explainable on other grounds. It is, however, to be stated that the stool examinations were started only on the 9th August, 1949, whereas the outbreak of cholera in the hospital was in progress from the 11th July, 1949 and had considerably declined by the time these investigations were undertaken.

During the course of an investigation of the effect of individual constituents included in Aronson medium widely used as a plating medium for the isolation of *V. choleræ*, it was found that the precipitation of rosaniline from alcoholic solutions of basic fuchsin by alkali was probably responsible for the inhibitory effects manifested in certain batches of the medium. Resistant colonies obtained in rosaniline media after a massive streak inoculation of cultures were seen to be predominantly rough. The minimum concentration of rosaniline needed to induce the rough change was found to be 0·03 per cent. Rough-smooth rough variants were derived from nine smooth races of *V. choleræ* with the aid of rosaniline as a dissociating agent.

3. Inquiry on cholera under the Director, School of Tropical Medicine, Calcutta.

The results of the investigation conducted are described under the following headings :—

I. *Value of slide examination of stools stained and unstained.*—Ten samples of stool from acute cases were examined. Films were made from these materials and were stained with carbol fuchsin (10 per cent) for 10 minutes and were examined for coma-shaped organism. Wet films were also made from these materials and examined for the presence of active motile organisms. The specimens were simultaneously cultured for bacteriological confirmation. The results show that seven out of ten samples were positive by culture, of these seven, five showed coma-shaped organism by staining and two showed active motility by wet-film preparation. The other three were negative by all these methods.

II. *Evaluation of different brands of peptone for the cultivation of vibrios.*—Fifteen recently isolated strains of *V. choleræ* were grown in four different brands of peptone water made with peptones of four different makes, namely Stern, U.S.P., bacto- and proteos-peptone. It was observed that the degree of growth differed with different brands and was the best with bacto-peptone.

III. *Relative value of the examination of rectal swab and fæcals specimen.*—Twenty samples of stools from 10 acute cases of cholera were collected by rectal swab as well as by catheter. Both the samples were examined bacteriologically. The results were as follows :—

Rectal swab.	Stool.	Number positive.	Per cent.
+	+	10	50
+	—	3	15
—	+	7	35

IV. *Direct plating versus peptone water enrichment of stools of acute cases of cholera.*—One hundred and thirteen samples of stools from 70 cases of cholera were directly plated on bile-salt agar and also inoculated in peptone water of pH 9·2 and then plated out on bile-salt agar after six to eight hours of incubation. The results were as follows :—

Direct plating.	Peptone water enhanced.	Number positive.	Per cent.
+	+	66	57·3
+	−	37	32·7
−	+	10	8·8

V. *Bandi's tube method.*—Some preliminary work was done on this method. Tubes tapering at the bottom designed after Bandi were made. These tubes contained 20 c.c. of peptone water and 1 c.c. of 1 in 160 dilution of cholera high-titre serum. The original titre of the serum was 1 in 6,400, so the final dilution was 1 in 3,200. Laboratory cultures of *V. choleræ* alone and also mixed with *B. coli,* were inoculated in these tubes. Fine granules were observed within four hours depending on the amount of inoculum. That these were due to clamping of vibrios was confirmed by microscopic examination of stained films of these granules. Epidemic in Calcutta subsided by the time the laboratory experiments were completed. So stools could not be examined by this method. Observations with cholera stool will be made during the next epidemic in Calcutta.

VI. *Effects of excretion of vibrios in stool under treatment with various drugs.*—Fifty-five cases were treated with sulphaguanidine, 38 cases with formocibazol and 21 served as controls. The results are given in a tabular form. Cholera vibrios were isolated from all these cases.

	Number of cases examined.	Persistence of cholera vibrios in days.						
		1	2	3	4	5	6	7
Sulphaguanidine-treated cases	55	23 or 41·8%	17 or 30·9%	6 or 10·9%	6 or 10·9%	2 or 3·2%	1 or 1·8%	*Nil*
Formocibazol-treated cases	38	15 or 39·4%	10 or 36·3%	6 or 15·7%	4 or 10·5%	3 or 5·2%	1 or 2·6%	*Nil*
Control cases	21	3 or 14·2%	4 or 19%	8 or 38%	3 or 14·2%	1 or 4·7%	1 or 4·7%	1 or 4·7%

VII. *Convalescent sera.*—Twenty-four samples of serum from 16 bacteriologically proven cases of cholera were collected for Dr. Bruce White and handed over to Dr. Krishnan.

VIII. *High-titre sera.*—About 75 c.c. of Inaba, Ogawa and Inaba rough high-titre sera were raised. Some of them proved unsatisfactory after absorption and most of them were used in the Bandi's tube method.

REPORT OF THE CHOLERA ADVISORY COMMITTEE.

Held in the Central Secretariat, North Block, New Delhi at 10-00 a.m. on the 19th November, 1949.

MEMBERS PRESENT.

Major-General S. S. Sokhey. (*Chairman*).
Lieut.-Colonel M. L. Ahuja.
Dr. R. B. Lal.
Dr. Satya Swaroop.
Dr. K. V. Krishnan.
Dr. C. G. Pandit.
Dr. S. R. Pandit.
Dr. K. V. Venkatraman. (*Secretary*).

Major-General A. C. Chaterji was unable to be present.

VISITORS.

Dr. S. S. Pillai.
Dr. P. M. Wagle.
Dr. S. C. Ghosal.
Dr. J. C. Gupta.
Dr. N. T. Advani.
Lieut.-Colonel S. D. S. Greval.
Colonel S. L. Bhatia.
Dr. R. Subramaniam.
Dr. A. M. Francis.
Dr. Gurkirpal Singh.

1. The Committee considered the work carried out by the following inquiries:—

(*i*) *Enquiry on cholera under the Director, Central Research Institute, Kasauli.*—Colonel Ahuja in presenting the report of the enquiry drew attention to the evidence obtained during the year for the confirmation of the previous finding that it is possible to isolate from Inaba and Ogawa strains of *V. choleræ* polysaccharide fractions of high antigenic value. He also referred to the attempts made to transform a fully rough vibrio into a smooth strain by repeated transfers in a peptone-water medium containing 10 per cent of a polysaccharide isolated from a smooth strain. In 20 such passages no serological change was observed in any of the hundreds of colonies tested and there was no evidence of any reversion of a fully rough variant to a smooth type. The investigation undertaken to see if the polysaccharide fraction of *V. choleræ* could produce anaphylaxis in passively immunized animals failed to reveal any evidence of inducing a state of anaphylaxis. In accordance with the recommendation of the Expert Committee of the WHO on Cholera, the preparation of monospecific serum was undertaken. Four hundred c.c. of sera are ready for transmission to Copenhagen. In conclusion Colonel Ahuja stated that further work which remains to be done on the immuno-chemical properties of vibrio polysaccharides could not be pursued until the services of a chemist with experience in immuno-chemical studies became available.

After discussion the Committee resolved to recommend to the Scientific Advisory Board the continuance of the enquiry only if the services of an experienced chemist of the type required could be made available to Colonel Ahuja for the purpose.

(ii) *Cholera enquiry under Dr. K. V. Venkatraman, Director, King Institute, Guindy, Madras.*—Dr. Venkatraman, while presenting the report of the enquiry, observed that the lines of investigation and the set-up of the organization that has been created at Trichinopoly for an investigation of the possibility of subclinical cases of cholera constituting a link between successive cholera outbreaks in the Cauvery delta had been fully discussed at the joint meeting of the WHO/OIHP/IRFA that had just concluded. Actual field work had been in progress only for a short time. A detailed survey of the selected villages has been completed and a systematic examination of fæcal and water specimens just commenced. Eight thousand seven hundred and eighty-eight stool specimens and 43 specimens of water so far examined have not yielded any isolations of *V. choleræ*. It was intended to continue this enquiry so that it may cover one complete year and the proposal submitted for continuance would cover the period up to the end of December, 1950.

Sir Sahib Singh Sokhey remarked that after attending the very interesting discussion on the subject at the joint WHO/OIHP/IRFA meeting where Dr. Venkatraman had given a fuller account of his work, he had come away with the impression shared by the WHO/OIHP Expert Group on Cholera and others present at that meeting that in this enquiry an excellent centre had been created for the investigation of many problems in the epidemiology of cholera. He was of the opinion that this fortunate circumstance should be exploited to the full on a long term basis to fill gaps in our knowledge and that the enquiry should be continued not for nine months only but for a longer period until all the outstanding problems were solved.

Dr. Lal referred to the work of a previous enquiry in the Diamond Harbour area which had a similar objective and desired to know how the present enquiry differed from the previous one. Dr. S. R. Pandit drew attention to the newer knowledge on the serology of the cholera group of vibrios and the improved technical methods that had become available in the intervening years and emphasized the desirability of exploiting the methods in a fresh study of the problem. Dr. C. G. Pandit observed that the results of the Diamond Harbour enquiry were available to the WHO Study Group when they met in Paris and that, after a careful study of the results, the Group desired that a further enquiry should be undertaken in a different area. They had the opportunity to visit the area now selected and acquaint themselves with the organization of the enquiry from which they expected very valuable results. The Director, OIHP had just handed over to him a further handsome contribution making a total of Rs. 40,000. At Dr. Pandit's suggestion the Committee resolved to record their appreciation of this handsome assistance.

The Committee finally resolved to recommend to the Scientific Advisory Board that the facilities now created at the centre be exploited on a long term basis for the investigation of problems relating to the epidemiology of cholera. The Committee left it to the S. A. B. to work out a suitable organization for the purpose.

(iii) *Cholera enquiry under the Director, School of Tropical Medicine, Calcutta.*—Dr. Ghosal in presenting the report of the enquiry stated that the following investigations had recently been started at the instance of Dr. Pandit: (*a*) Studies in relation to the evaluation of different brands of peptone for the cultivation of vibrios, (*b*) on the relative value of the examination of rectal swab and fæcal specimens in the diagnosis of cholera, (*c*) the comparative efficacy of direct plating versus peptone-water enrichment of stools of acute cases of cholera and (*d*) the value of Bandi's tube method in the early diagnosis of cholera have been started. He asked for the continuance of the enquiry during the coming year to complete these investigations and the Committee resolved to recommend to the Scientific Advisory Board the continuance of the enquiry.

(*iv*) *Enquiry into the endemicity of cholera in India under Dr. Satya Swaroop, Professor of Statistics, All-India Institute of Hygiene & Public Health, Calcutta.*—Dr. Swaroop stated that the object of the enquiry was to demarcate areas endemic for cholera in India, to study the factors contributing to the endemic status of an area and to collect statistical information bearing on endemicity of the disease in different areas. Preliminary work on district figures had been completed and broad groups of districts in which endemic foci of cholera are likely to fall had been demarcated. He proposed to continue the study on figures of smaller divisions more exactly to define the real endemic areas. In this connection he stated that the provision made in his budget proposal for the employment of a computor on the minimum of the scale was found to be inadequate and requested that a provision of Rs. 200 per month be made to enable him to obtain a competent assistant. The Committee supported this request and commended to the Scientific Advisory Board to sanction it.

(*v*) *Cholera research work under Dr. K. V. Krishnan, All-India Institute of Hygiene & Public Health, Calcutta.*—Dr. Krishnan in presenting the report of the work done since September 1949, relating to (*a*) the study of agglutinin response to anti-cholera inoculation in normals, vaccinated persons and recovered cholera cases with a view to finding if it could be used in the retrospective diagnosis of cholera, (*b*) study of cholera vibrios in natural waters in Calcutta and (*c*) the study of the mutation of cholera and other vibrios, stated that the results so far obtained were promising but that no conclusion could be drawn at this stage of the work. Of 75 samples of water examined from three different sources, six showed the presence of *V. choleræ* and two showed El Tor vibrios. The technique of isolation that proved most efficient was the Kieselguhr method. From a study of the agglutinin response after inoculation it would seem that normal sera of persons do not usually have any agglutinating action on suspensions of the cholera vibrio and that the majority of sera of vaccinated persons do not give positive agglutinin above 1 in 80. Sera of cholera convalescents generally give positive agglutination well over 1 in 320 and when vaccinated give much higher titres. He proposed to continue the work in the three directions already mentioned and also take up work on cholera toxins during the coming year. Colonel Ahuja and General Sokhey suggested that in addition to agglutination response, precipitin reaction and vibrio-killing power of the serum might be tried. The Committee resolved to recommend to the Scientific Advisory Board the continuance of the enquiry.

2. The Committee considered the proposal for a new enquiry on the thorough study of stools of patients with symptoms of cholera by Dr. H. N. Chatterjee at the National Medical Institute, Calcutta. It was noted that some of the lines of the proposed study were already covered by the existing enquiries and that the other outlines were vague. The Committee, therefore,

did not recommend to the Scientific Advisory Board the proposal for the new research scheme on cholera.

3. The Committee recorded the extracts from the minutes of the ninth meeting of the Medical Research Liaison Committee for India held on the 2nd March, 1948, regarding :—

(*a*) Notes by Sir John Taylor and Mr. P. Bruce White on their deputation to Egypt in connection with the outbreak of cholera in autumn 1947.

(*b*) Comments on the report of the Cholera Advisory Committee of the Indian Research Fund Association held at Delhi in December, 1947.

MALARIA.

1. Insecticide and mosquito repellent inquiry under the Director, Malaria Institute of India, Delhi.

Studies on different insecticidal formulations were continued both in the laboratory and the field in Delhi and Coonoor.

Delhi laboratory.—

(*a*) Studies were continued to improve the emulsion concentrate prepared in Aromex as solvant. The solubility of DDT in benzene at laboratory temperatures was found to be over 100 per cent (w /v), and it may be possible to produce a highly concentrated stock emulsion with this solvant, but it is highly volatile and inflammable. This factor would naturally restrict its use a great deal unless it can be mixed with a less volatile solvant or to treat it in some manner so as to render it less volatile. In this respect prospects of using chlorinated benzene are being studied. Different formulæ were tried using aromex, turpentine and xylol in combination with benzene. Fifty per cent emulsion concentrates prepared with benzene-turpentine and benzene-aromex combinations using oleorosin soap as emulsifier have proved promising. If chlorobenzene becomes available in large quantities the problem of solvants for DDT will be solved for some time to come. Work on the various formulations is in progress and the formulæ found satisfactory will be tried out in the field during the next malaria season.

(*b*) Attempts are being made to prepare wettable powders of DDT, similar to those obtainable in the market. Various light inert substances, such as soapstone powder, kaolin and chalk, have been tried. DDT was ground with these diluents in the presence of dry soap powder (Lux or Tomco) to as fine a consistency as possible in a mortar. The best results were obtained in a combination of DDT 50 per cent, kaolin 45 per cent and soap 5 per cent. Experiments are being continued. When a satisfactory formula is evolved, correct degree of pulverization can be ensured by ball mills.

Delhi field.—

1. Trials were carried out in the field with preparations of Gammexane both as a larvicide and as a residual insecticide spray. The following preparations supplied by the Imperial Chemical Industries, Ltd., were used :—

(*a*) D.025 (gamma isomer content 0·6 per cent) as a larvicide and
(*b*) P.520 (gamma isomer content 6·5 per cent) as a residual indoor spray.

(*a*) *Larvicides :* Three large breeding places covering an area of about 10 acres were selected for these trials. The prevailing density of larvæ in these breeding places, and the density of adult mosquitoes in the village lying in between these breeding places were determined. The breeding places were treated with D.025 at the rate of 5 lb. diluted with 45 lb. diluent per acre. About 80 to 90 per cent reduction in the density of breeding was observed within 24 to 48 hours after dusting. Fresh breeding commenced within 5 to 6 days and went on unhampered. The density of mosquitoes in the village remained unaffected by these larvicidal operations.

(*b*) *P. 520 as a residual spray :* P.520 is a water-dispersible preparation, to be mixed with water at the rate of 12 ounces per gallon (7·5 per cent) and sprayed at one gallon per 2,000 sq. ft. A village (Azadpur) was sprayed (on 1-3-1949) with this preparation at the above dose depositing 11 mg. gamma isomer per sq. ft. Another village (Hyderpur) which was not sprayed served as comparison. In Azadpur the number of mosquitoes which was 20 per catching station was reduced after the spraying by 95 per cent and remained at that level for about 12 days. Then it slowly began rising reaching 11, i.e. (50 per cent of the original level) on the 18th day after spraying. Collection from the control village was quite high throughout.

Village Jogabai, with a very high density of mosquitoes, was sprayed with P.520 in the same manner as above (on 21-4-1949). The number of mosquitoes continued to remain low (less than 10 per cent of the original collection) for over three weeks when the collection of mosquitoes had to be suspended due to natural decline in their prevalence as a result of adverse weather conditions.

2. Experiments are in progress to determine the minimum single dose of DDT that will be enough to give protection for the whole season. Previous years' results have shown that a dose of 50 mg. DDT per sq. ft. is enough to give protection for about six weeks. Doses of 100 mg. and 200 mg. per sq. ft. of wall surface were sprayed in three villages while a fourth village was left untreated, to serve as a control. The emulsion prepared with aqueous extract of '*Rittha*' (dhobi nut) and the suspension prepared with gelatine and gum acacia were used to spray these villages.

In all these villages preliminary collections of mosquitoes from fixed catching stations were made for a period of three to four weeks to ascertain the prevailing density of Anophelines. Spraying was carried out only when the mosquito collections remained consistently high, indicating that the effect of previous sprays, if any had been applied, was not present any more. The interval between the previous spraying and the present experiment was always sufficiently long to avoid any chances of re-activation of the old sprays.

One village (Jogabai) was sprayed (on 6-8-1949) with a 5 per cent DDT emulsion with *Rittha* as emulsifier, at 4 c.c. (200 mg. DDT) per sq. ft., a second village (Masigarh) was sprayed (on 13-8-1949) with the same emulsion at 2 c.c. (100 mg. DDT) per sq. ft., while a third village (Timarpur) was sprayed (on 29-8-1949) with the 5 per cent suspension at 2 c.c. (100 mg. DDT) per sq. ft. The collections of mosquitoes has so far remained between one to five per cent of the original collections, 30 to 35 days since first two villages were treated. In the third village which was sprayed 15 days later the collections were ranging between 10 and 15 per cent of the original collections. The prevalence of mosquitoes in the control village has remained high throughout. Observations are being continued.

Two preparations of gammexane (benzene hexachloride) D.025 (gamma isomer content 0·6 per cent and S.G.034 (gamma isomer content 3·0 per cent) received from the Imperial Chemical Industries (India), Ltd., were tested in the laboratory for their efficacy in controlling the breeding of flies. Trays containing sterile horse dung medium were inoculated with a known number of laboratory-reared maggots and dusted over with these preparations at the recommended dose. Both these preparations have shown inhibitory effect on the breeding of flies. The above findings will have to be confirmed by further large-scale trials in the field.

Samples of "Smokoil" were tested for their mosquito repellent effect. A number of new designs of equipment used in malaria control were also tested during the period.

Coonoor laboratory.—

Laboratory experiments were carried out to improve on the 70 per cent solid emulsion to avoid the 'buttering' of the DDT. It was found that the incorporation of 10 per cent by weight of caustic soda or caustic potash in the said preparation helped to prevent this buttering. This amount of alkali did not affect the toxicity of the DDT.

Laboratory trials to improve the method of preparing the suspension of DDT are also in progress.

A colony of *C. bitæniorhynchus* has been successfully established.

Camphor-yielding *Ocimum* plants have been successfully cultivated for testing their repellent effect on mosquitoes.

Coonoor field.—

1. With a view to study whether, other factors remaining the same, a mere change in the distribution of the spray will make any difference in the prevalence of mosquitoes, one hut was sprayed with 1·25 per cent spray at 2 c.c. per sq. ft., while another was sprayed with 2·5 per cent spray at 1 c.c. per sq. ft. The latter gave a patchy spray due to the limited quantity though the dosage remained the same (25 mg. DDT per sq. ft. in both cases). Observations showed that patchy spraying afforded appreciably less protection; spraying uniformly is essential to get the maximum benefits of residual spraying.

2. *Spraying with gammexane.*—The preparation P. 520 (I.C.I.) containing 6·5 per cent gamma isomer was used. A 10 per cent suspension of this preparation in water sprayed to give a deposit of 15 mg. gamma isomer per sq. ft. remained effective for 63 days. When the dose was doubled (30 mg. gamma isomer per sq. ft.) the effect lasted for over 96 days.

3. DDT applied as a 2·5 per cent suspension prepared either according to the M.I.I. formula or the 50 per cent wettable powder at a dose of 2 c.c. (50 mg. DDT) per sq. ft. gave protection for more than 97 days.

With DDT emulsion, sprayed at the same dose, no mosquito has been collected for a period of 60 days. Observations are being continued.

A 2·5 per cent DDT spray prepared from the 70 per cent solid emulsion concentrate was sprayed at the rate of 2 c.c. (50 mg. DDT) per sq. ft. Except for one infected *A. fluviatilis* found on the 38th day after spraying, the mosquito incidence in the sprayed huts has been of no significance up to now (82 days since spraying). Observations are being continued.

2. Inquiry on malaria under Dr. R. N. Chaudhuri at the School of Tropical Medicine, Calcutta.

CHEMOTHERAPY AND CHEMOPROPHYLAXIS OF MALARIA.

Comparison of action of paludrine, chloroquine and camoquin in equivalent single doses.—This investigation was undertaken with a view to devise a simple form of mass treatment to suit people in rural areas, who are generally unable to take a full course of treatment. It was intended to determine whether a single dose (0·5 g.) of each of these three drugs could effect a clinical cure. Trials were given in 75 patients admitted into the hospital. These included 36 *falciparum*, 35 *vivax*, 2 mixed (*falciparum* and *vivax*) and 2 *malariæ* infections.

Results.—Clinical cure was effected in all the cases; the asexual parasites disappeared from the peripheral blood within three days after the treatment was started in 80 per cent of those treated with paludrine and 100 per cent of those who were given chloroquine and camoquin and the fever was controlled in 84 per cent, 100 per cent and 100 per cent of the cases, respectively. All the chloroquine and 80 per cent of camoquin cases became parasite-free by the end of the second day, and 84 per cent of the former and 76 per cent of the latter became afebrile. Thus, all the three drugs are effective in bringing about a clinical cure, chloroquine being quick, paludrine slow and camoquin intermediate.

The patients were watched for 1 to 2½ months after treatment in hospital. None of them had any further anti-malarial treatment after the single dose they had received on admission. During this period nine out of 25 patients treated with paludrine had recurrence of fever. Three of them who had *vivax* infection on admission relapsed with homologous parasites in the blood within a fortnight after treatment while still in the hospital; the remaining six had after discharge clinical malaria (blood was not examined) within the period of observation. Five amongst 25 camoquin series had recurrence of fever after the treatment was given. Three patients, two due to *falciparum* and one due to *vivax* infections relapsed (homologous parasites being found), after 15, 13 and 27 days, parasites appeared temporarily with no fever while they were still in the hospital; the other two had recurrence of fever after 1 and 2½ months (the blood not examined). Two patients with *vivax* infection in 25 chloroquine series had 'relapsed' (parasites found) after three and four weeks. These results, therefore, indicate that the destruction of asexual parasites together with the fall of temperature can be effected by a single dosage of 0·5 g. of any of these drugs. These may be used for treatment of malaria cases in the endemic areas where no better means of treatment is possible. Relapse and re-infection, however, will naturally take place. They can be adequately dealt with in the light of the report of further work in this line.

Paludrine and plasmochin in vivax *malaria.*—Sixteen *vivax* cases have so far been treated with paludrine 0·3 g. and plasmochin 0·01 g. both combined and given twice daily for 10 days. In 94·7 per cent of cases the asexual parasites disappeared from the peripheral blood and in 79 per cent the temperature became normal within three days after the institution of the treatment. Only one patient had clinical malaria (blood not examined) after two months. Two patients, not included in this series, could not take the full course owing to vomiting in one and severe palpitation with depression in the other.

Clinical trials with paludrine.—Further trials were carried out in addition to those already reported last year with two regimes, i.e. a 10-day course and a 4-day course.

Ten-day course.—This was tried in 45 indoor patients: (*a*) 25 cases (13 *vivax* and 12 *falciparum*) having 0·1 g. t.d.s. and (*b*) 20 cases (6 *vivax* and 14 *falciparum*) having 0·3 g. B.D. Asexual parasites disappeared from the blood within three days after the treatment was started in 92 per cent of (*a*) group and in 95 per cent of (*b*) group. The corresponding figures as regards temperature were 84 and 95 per cent, respectively. Gastric irritation (nausea or vomiting) was noticed in a few patients towards the end of the (*b*) course, and on this account the treatment was discontinued in one patient who, however, has not been included in the above series. One patient of the (*b*) group had malaria with homologous infection three weeks after the course was completed.

Four-day course.—Eighteen patients (14 *vivax* and 4 *falciparum*) were treated with paludrine, 0·6 g. in two doses on the first day, and 0·3 g. in one dose on subsequent three days, the total quantity being 1·5 g. The blood became parasite (asexual) free in 100 per cent and temperature became normal in 88·8 per cent within three days after the treatment was started. Among them only nine patients were closely observed as they belonged to the staff of this Institution. None of them had yet relapsed though three months have already passed. This four-day course is being used on our recommendations, by some physicians in the Medical College and Lake Medical College Hospitals, and almost identical results have been encountered.

(*a*) Falciparum *infection refractory to paludrine.*—A boy aged 12 with M.T. infection, who never had paludrine in his life, was given 0·6 g. of the drug on the first day followed by 0·3 g. daily for the next eight days. There was no effect on fever or on the parasites. After four days paludrine was repeated (four-day course mentioned above) but still without any appreciable effect. Both blood and urine gave strong positive reaction to paludrine. His blood was inoculated to a volunteer, and immediately thereafter he was given a single dose of chloroquin 0·5 g. The response was immediate. Three days later the volunteer was given paludrine 0·3 g. daily for four days. Nevertheless, he developed fever 17 days after inoculation and the blood showed M.T. rings. He was again put on paludrine 0·3 g. B.D. which was continued for eight days. There was little or no effect, and the drug was discontinued. Both blood and urine gave strong positive reaction to paludrine. On the following day he was given chloroquin, 0·5 g. after his blood was inoculated to another individual. There was no doubt a positive response but not so quick as in the previous patient. The latter developed malaria after 15 days and responded to paludrine (0·2 g. B.D. for 10 days) five days after the treatment was started.

The case illustrates an instance of natural resistance of a strain of *falciparum* parasite to paludrine, although there was positive response though slow in the second volunteer.

(*b*) *A fatal case.*—An old man with history of fever and diarrhœa for eight days came walking to the hospital. The blood showed heavy M.T. infection, the number of parasites being 1,093,440 per c.mm. Paludrine lactate was given intravenously and 2 g. intramuscularly on admission. This was followed by three more injections, 0·1 g. each, the first at intervals of four hours and the third, of six hours along with palliative therapy. On the second day blood showed a few M.T. rings but the patient was drowsy. He was given another injection of paludrine 0·3 g. intravenously at 11 a.m. and was put on 0·2 g. t.d.s. by mouth. He was given a plasma transfusion and stimulants, etc. Towards the evening the condition further deteriorated; hiccough started; the patient became comatose and died at night, i.e. 34 hours after admission.

Post-mortem examination showed capillaries of brain, heart and intestine packed with parasitized red cells. Heart's blood showed clump of nucleated cells—apparently myeloblasts.

The case illustrates that despite 1·5 g. of paludrine given by injection in little over 24 hours it had little or no effect on proliferation of the parasites in the internal organs, although the parasites in the peripheral blood were markedly reduced.

Clinical trial of neochin.—Sixteen indoor patients were treated with neochin, they included seven M.T., six B.T., one Q. and two mixed M.T. and

B.T. cases. The dosage recommended by the manufacturers, one tablet gr. 5 b.d. for two days and then one daily for two days having been found unsatisfactory, was changed to one tablet t.d.s. for four to five days with which 13 patients were treated. The average period by which the temperature became normal and blood parasite (asexual) free was 3·5 and 4·1 days. Seven out of 11 patients kept under observation for 14 to 48 days relapsed after an average interval of 14 days after completion of treatment (5 M.T. and 2 B.T. cases) when they were treated with different anti-malarial drugs. No untoward symptom was noticed.

Paludrine level in blood.—The hydrolytic method of Spinks and Tottey (1946) was employed. Owing to difficulties encountered to standardize the technique, estimations have so far been made only in a small number of cases. With single doses of 0·6 g., 0·5 g. and 0·3 g. the average whole blood concentration attained the maximum of 1,354, 1,184 and 725 micrograms per litre respectively usually after four hours. It fell rapidly on the second day and generally disappeared on the fourth day. Individual variations were also observed.

FIELD STUDY OF CHEMOPROPHYLAXIS OF MALARIA.

The experimental work on the control of malaria in a selected hyperendemic rural area of West Bengal (Hooghly district) with weekly or bi-weekly doses of paludrine, chloroquine and camoquin was carried out from July to December, 1948.

State of health.—In the initial survey (May 1948) among 1,458 individuals the spleen and parasite rates were 62·8 per cent and 10·7 per cent, respectively. The nutritional state was poor, clinical signs of nutritional deficiency being evident in 44·4 per cent of the population examined. The hæmoglobin value, estimated in 1,097 individuals by the Adams-Talquist scale, was found to be below 50 per cent (7·8 g.) in 13 per cent, between 50 and 70 per cent (7·8 g. to 10·9 g.,) in 70 per cent and over 70 per cent (10·9 g.) in 17 per cent. The total serum protein determined in 61 individuals by the copper-sulphate method, however, showed almost normal values.

Method of work.—The population of eight villages was divided into five groups of about 250 persons in each, four for chemoprophylactive investigations and one for the control. The prophylactic doses were as follows :—

Group.	Drug and frequency of administration.	Above 10 years.	4 to 10 years.	Below 4 years.
I	Paludrine twice weekly	0·1 g.	0·05 g.	0·03 g.
II	Paludrine once weekly	0·3 g.	0·2 g.	0·1 g.
III	Camoquin once weekly	0·2 g.	0·1 g.	0·05 g.
IV	Chloroquine once weekly ...	0·25 g.	0·125 g.	0·62 g.
V	Control—Quinine was given only in cases of malarial attacks.			

Malaria was already prevalent when treatment was started in July, but no drugs were specially given even to control febrile attacks. The parasite rate was determined at intervals of two months during the active malaria season and afterwards at intervals of three months.

Results.—The *infection rate* per 100 at different stages is shown in the Table below. The number of subjects were not the same each time as some of them refused blood examination :—

TABLE

Infection rate per 100 examined at different stages of the experiment. The figures within bracket indicate the number of subjects whose blood was examined.

Group.	May 1948.	September 1948.	November 1948.	February 1949.	May 1949.
I	12 (235)	6 (222)	4·48 (223)	1·36 (221)	4·10 (167)
II	9·42 (276)	9·13 (219)	5·73 (227)	3·09 (259)	9·56 (240)
III	10·57 (246)	8·02 (212)	1·89 (212)	0·63 (159)	5·95 (84)
IV	7·9 (227)	5·9 (101)	2·6 (193)	1·6 (241)	7·37 (190)
V	11·32 (265)	15·03 (173)	36·87 (179)	14·57 (199)	17·4 (224)

There was a definite progressive decline in the parasite rate in all the groups except the control. It should be recorded that difficulties were at first encountered ; while in Group I the distribution of drugs was generally regular from the beginning, in other groups, there was some irregularity in this respect, especially during the earlier period owing to lack of co-operation among a section of the population and counter-propaganda by interested persons. Also some persons (Group II) found it difficult to swallow three tablets of paludrine at a time and actually vomited these out. These difficulties were eventually overcome and striking result were noticed in November. The malaria transmission is usually at its highest peak during October to November. The parasite rate in the control group rose from 11·3 per cent in May 1948 to 37·1 per cent in November 1948, while it declined to 4·48, 5·73, 1·89 and 2·6 per cent in Groups I, II, III and IV respectively. In the natural course malaria declines during winter, and this accounts for further fall in February 1949 which was noticeable even in the control group.

The four sets of observations (September 1948, November 1948, February 1949 and May 1949) for Groups I to IV were statistically analysed and the results show that in May 1949 and September 1948, the *infection rates* were generally high for all the four treatments, low in November 1948 and considerably low in February 1949. The seasonal fluctuation shown by the four groups are more or less same. Régimes I, III and IV are more or less equally effective, but II shows consistently higher rate than the remaining three but significantly lower rate than the control. In control Group V the infection rate is much higher than any other group in all the stages of the experiment from September 1948 to May 1949. Statistical analysis confirms this point, and in November when the control group shows the maximum infection rate the treated groups record practically the least. Another point worth noticing is that in the control group a significant number of subjects who had parasites in the blood in September 1948 continued to be positive up to February 1949. There was a general rise in the parasite rate in May, but this cannot entirely, if at all, be due to the post-suppression effect of the drugs, for the rise was also noticeable in the control group.

The spleen rate was determined in February 1949 which showed a marked reduction as will be seen from the following. This reduction was more noticeable in the camoquin and chloroquine groups (III and IV). There was some reduction in the control series due to usual seasonal variation, but this is not of much statistical significance.

Group.	I	II	III	IV	V
May 1948 ...	55·4	72·8	64·2	57·7	61·2
February 1949 ...	24·4	35·6	16·4	18·2	50·6

As regards *fever* it was our general impression, supported by voluntary statements of the people themselves, that those who took the drugs regularly had very little malaria, while many of the control group were badly suffering from it. It was, however, observed that some persons, though showing asexual parasites in the blood, were apparently free from fever and carrying on their usual work.

No toxic symptom was encountered in any group except a small number of persons who felt sick after paludrine for a short while. A few cases, however, responded poorly to paludrine.

In spite of the favourable results, no conclusion should be drawn at this stage. Malaria is a disease which may vary in intensity from season to season, and one year's observation cannot be sufficient for the test.

The administration of drugs has again been commenced from July 1949 and, as before, without any anti-mosquito measures. A notable feature this time is the greater co-operation of the people. Up to 15th September, 1949 the results are as follows: Chloroquine is leading in efficiency, paludrine, 0·3 g. (in one tablet) once a week is giving much better results than before, but the effect of 0·1 g. twice a week is so far below the last year's results.

3. Inquiry on the chemotherapy of malaria under Dr. K. Ganapathi at the Haffkine Institute, Bombay.

Synthesis of compounds of potential anti-malarial activity.—During the course of period under report a large number of compounds of probable anti-malarial activity have been prepared, which compounds will now be subjected to prophylactic tests with regard to inhibition of development of sporozoites, etc., in the infected mosquitoes and to suppressive activity tests in chicks infected with *P. gallinaceum.*

In view of the reported anti-malarial activity of some sulphones, such as diazone, about 40 sulphone derivatives were synthesized involving many active groups, known to be associated with therapeutic activity. About ten of these compounds were tested for the prophylactic activity in the mosquito but with negative results under the dosages so far tried while testing two of the same for suppressive activity against *P. gallinaceum* infection in chicks. It has been noted that one of the compounds appeared to give encouraging results although it is too premature at this stage to pass a final verdict. Further work in this direction is under progress.

The second series of compounds prepared comprises of di-substituted amines, mono- and di-substituted thioureas, and a few di-thiobiurates. A total of about 45 compounds have been synthesized and now that the technique of prophylactic tests in the mosquito host has been more or less standardized, it is contemplated that in the first instance all the above referred to 80 to 85 compounds should be subjected to tests for prophylactic activity as indicated above.

It is earnestly hoped that if the question of supply of chicks is satisfactorily solved, it will be possible to subject the above compounds to tests for suppressive activity. It is also conjectured that some of these compounds may show promising suppressive activity worthy of subsequent detailed study.

Further synthetic work connected with new routes for the synthesis of anti-malarial compounds of established reputation are under way and attempts are being made also to develop and synthesize many more compounds of possible anti-malarial activity.

Testing of anti-malarials.—The chicks that have been used so far were incubator-hatched from commercially obtained eggs, the fertility rate of which is low and variable. These chicks are half the weight of breeds used for this work in standard procedures. A suitable inoculum had, therefore, to be worked out. Inocula of 8 million to 24 million parasitized red cells were tried and 16 million was found to be a convenient density, producing a readily detectable infection (0·1 per cent) on the day after inoculation, with a count of not less than 50 per cent cells infected on the fifth day. Survival of untreated birds after the seventh day is uncommon.

Chloroquine, synthetized at this Institute, and two new quinoline compounds have been tested in dosages based on the known quinine equivalents of similar compounds. Chloroquine showed activity. One quinoline compound, Q15, also appears to be active in a higher dosage than the one first tried. Further trials would be necessary to confirm this.

Recent work in the U.S.A, shows that a definite relation does exist between drug activity in the infected mosquito and drug activity in the sporozoite-induced infection in the vertebrate, thus providing a new approach in the search for prophylactic drugs. Accordingly, we started testing drugs against *P. gallinaceum* in *Ædes œgypti*. It was found that 0·02 per cent paludrine synthetized at this Institute completely inhibited gland infection when incorporated in the diet of the mosquitoes after the infective blood meal. This drug will thus serve as standard for comparison. Twelve quinoline and sulphone compounds were tested by this method in solutions of maximum solubility in water. Q15 first gave indication of activity in this test but owing to its low solubility consistent results could not be obtained. This raises the problem of administering insoluble drugs to mosquitoes. Artificial blood meals by known membrane-feeding techniques offer some hope. Locally obtained goldbeaters' skin and chick skins have been tried, but without much success. A sample of goldbeaters' skin has just been received from the U.K. and this is giving excellent results. The main problem now is to administer the drugs as solutions or as suspensions in blood plasma or other medium.

4. Inquiry on complement-fixation test in malaria for the assessment of anti-malarial drugs under Dr. J. C. Ray, at the Indian Institute for Medical Research, Calcutta.

I. *Strains.*—Three healthy *rhesus* monkeys were injected both intravenously and intramuscularly with the blood containing *P. knowlesi*, *P. cynomolgi* and *P. inui* parasites. On and from the third day after inoculation blood slides of the three monkeys were examined. The monkey No. 2N infected with *P. knowlesi* showed presence of parasites in r.b.c. but the other two showed negative results. Examination of smears was continued daily up to 23rd June, 1949, during which period monkey 2N only showed infection, others failing to take up infection. All forms of parasites, viz. rings, trophozoites and schizonts, were seen but gametocytes were found to be very scanty. Total parasite count varied from one to two per cent only in terms of r.b.c. count. Transmission of infection from monkey 2N to other clean monkeys failed.

Attempts were then made to increase the virulence of the strains received by two methods :—

A. By splenectomy or ligature of splenic artery.

B. Through mosquito infection.

A. Splenectomy was done on monkey No. 2N on 23rd June, 1949 and examination of blood smears was continued from 25th June, 1949 till 31st August, 1949 when the monkey died. During this period of one hundred days, the parasite count rose to 35 per cent on 2nd July, 1949 (i.e. on the ninth day of infection) and then gradually the count diminished. Two clean monkeys No. 7N and 8N were operated on for ligature of splenic artery and splenectomy on 8th July, 1949 and 14th July, 1949, respectively. They were injected both intravenously and intramuscularly with infected blood from the splenectomized monkey No. 2N. Blood smears from monkeys No. 7N and 8N were examined up to 25th August, 1949 but they showed no infection.

B. Since transmission of infection to clean monkeys failed even after splenectomy, and due to rapid decrease in parasite count in monkey No. 2N, an attempt was made to increase the virulence of the strain through mosquito infection. On 23rd July, 1949 a few laboratory-bred female *A. annularis* mosquitoes were fed on monkey No. 2N when its parasite count was found to be only 10 per cent with a few gametocytes. The same batch of mosquitoes were fed twice again at intervals of six and eight days. On 14th and 19th days after the last feeding several mosquitoes were dissected for detection of the infection in the salivary glands. No infection was, however, observed in mosquitoes on both these occasions. Monkey No. 2N died on 31st August, 1949.

II. *Preparation of anti-monkey hæmolysin.*—In the preparation of anti-monkey hæmolysin by injection of normal saline suspensions of washed monkey r.b.c. in rabbits the following three methods were followed :—

(*i*) The dose being fixed, percentage of r.b.c. emulsions were increased, e.g. the dose being fixed at 2·5 c.c. the percentage of r.b.c. emulsion varied from 5, 10, 15 and 20 per cent. Injections were given daily in a healthy rabbit intravenously, total number of injections being four.

(*ii*) Percentage being fixed at 50, dose varied from 1 c.c. to 5 c.c. with the increment of 1 c.c. This was injected intravenously daily for five days.

(*iii*) Dose and percentage being fixed at 3 c.c. and 5 per cent respectively, 14 such injections were given intravenously on consecutive days for a fortnight.

In the serum of the rabbit in experiment (*i*), the titre of amboceptor rose to 1 in 50, whereas that in experiment (*ii*) no hæmolysin was obtained even in 1 in 50. The hæmolysin titre in serum of rabbit used in experiment (*iii*) however, rose as high as 1 in 200.

Preparation of an anti-monkey hæmolysin with a batch of 12 rabbits following the above method with slight modification in the dosage is in progress.

For want of virulent strains of ***Plasmodium knowlesi***, it has not been possible to proceed with the work along the lines indicated in the scheme. Attempts are being made to obtain virulent strains from abroad.

REPORT OF THE MALARIA ADVISORY COMMITTEE

Held in the Committee Room 'B.' Central Secretariat, North Block, New Delhi at 10 a. m. on the 21st November, 1949.

MEMBERS PRESENT.

Lieut.-Colonel Jaswant Singh. (*Chairman*).
Dr. A. C. Banerjea.
Brevet-Colonel R. N. Chopra.
Dr. K. Ganapathy.
Dr. M. O. T. Iyengar.
Dr. B. Ananthaswamy Rao.
Dr. D. N. Roy.
Dr. D. K. Viswanathan.
Dr. K. S. Viswanathan.
Dr. I. M. Puri. (*Secretary*).

VISITORS.

Dr. A. K. Adhikari.
Dr. N. T. Advani.
Dr. R. K. Anderson.
Lieut.-Colonel J. Hay Arthur.
Mr. R. S. Bery.
Major-General S. L. Bhatia.
Mr. Paul Bierstein.
Group-Captain D. N. Chakravarti.
Dr. T. Chandrasekharaiya.
Dr. R. N. Chaudhuri.
Dr. S. Dakshinamurty.
Dr. C. R. Das Gupta.
Dr. S. L. Dhir.
Lieut.-Colonel A. N. Duggal.
Dr. A. B. Gilroy.
Dr. J. C. Gupta.
Dr. C. S. P. Hamilton.
Dr. Peter C. Issaris.
Major S. L. Kalra.
Lieut.-Colonel C. C. Kapila.
Dr. V. R. Khanolkar.
Dr. A. K. Krishnaswami.
Lieut.-Colonel C. K. Lakshmanan.
Dr. Daulat Ram Mehta.
Dr. Dev Raj Mehta.
Lieut.-General K. S. Master.
Colonel S. Narain.
Dr. R. Pal.
Dr. E. J. Pampana.
Dr. C. G. Pandit.
Dr. S. S. Pillai.
Dr. C. K. Prasada Rao.

Dr. M. V. Radhakrishna Rao.
Major N. G. S. Raghavan.
Dr. T. S. Rama Rao.
Dr. K. P. Rao.
Dr. T. R. Rao.
Dr. N. S. Sethi.
Dr. P. Sen.
Dr. R. S. Srivastava.
Dr. R. Subramaniam.
Major R. Subramanian.
Dr. K. V. Venkatraman.
Dr. E. B. Weeks.
Major C. P. Nair.

PROCEEDINGS.

The report on the inquiries carried out during the current year and proposals for 1950-51 were considered.

I. EXISTING INQUIRIES.

1. *Insecticide and mosquito repellent inquiry under the Director, Malaria Institute of India, Delhi.*—Lieut.-Colonel Jaswant Singh giving a brief account of the work done during the year stated that studies were continued to improve the emulsion concentrates prepared in medium-kerosine-extract (Aromax).

Solubility of DDT in benzene, available in large quantities in this country, at laboratory temperatures to produce a highly concentrated stock emulsion was found to be over 100 per cent (w/v) but owing to its highly volatile and inflammable nature, possibility of using chlorinated benzene instead was being explored. Different formulæ were tried and 50 per cent concentrates prepared with benzene-turpentine and benzene-aromax combined with oleorosin soap as emulsifier have given encouraging results.

Attempts have also been made to prepare DDT wettable powder similar to those obtainable in the market. A combination of DDT 50 parts, kaolin 45 parts and soap 5 parts has so far yielded the best results.

Trials were also carried out in the field with the following preparations of benzene hexachloride (gammexane) both as a larvicide and as a residual insecticide spray :—

(*a*) D.025 (gamma isomer content 0·6 per cent) as a larvicide and

(*b*) P.520 (gamma isomer content 6·5 per cent) as a residual indoor spray.

Three large breeding places covering an area of about 10 acres were selected and treated with D.025 powder at 5 lb. diluted with 45 lb. diluent per acre. About 80 to 90 per cent reduction in the density of *Anopheline* breeding was observed within 24 to 48 hours after dusting but breeding of mosquitoes reappeared within 5 to 6 days.

Two villages were selected for residual spraying experiment with P.520. (1) Azadpur was sprayed and another village Hyderpur was left unsprayed for comparison purposes. As a result of spraying 11 mg. gamma isomer per sq. ft. in Azadpur, the number of mosquitoes was reduced by 95 per cent and remained at that level for 12 days, after which the number began to rise and reached 50 per cent of the original level on the 18th day. Collections from the comparison village remained high throughout. (2) Another village (Jogabai) was also treated with the P.520 (21-4-1949) and the number of mosquitoes was reduced to 10 per cent and remained at that level for over three weeks when the collection was suspended due to a natural decline in the density of mosquitoes in the unsprayed village.

Experiments were in progress to determine the minimum dose of DDT effective for the whole season.

Two villages (Jogabai and Masigarh) were sprayed on August 6th, 1949 with a 5 per cent DDT emulsion with *Rittha* as emulsifier, at 4 c.c. (200 mg. DDT) and 2 c.c. (100 mg. DDT) per sq. ft., respectively, while a third village (Timarpur) was sprayed with 5 per cent suspension at 2 c.c. (100 mg. DDT) per sq. ft. After 80 to 85 days, collection of mosquitoes was observed between 1 and 5 per cent of the original collection in the first two villages, while it was 10 to 15 per cent in the third village. Jogabai had been treated on a previous occasion (21-4-1949) with gammexane P.520, but since then the mosquito density had gone up to a high level within a few weeks.

Two preparations of gammexane D.025 and S.G.034 were tested in the laboratory for their efficacy in controlling the breeding of flies. Both these preparations showed inhibitory effect but large scale trials in the field are still required.

Samples of " Smokoil " for their mosquito repellent effect and new designs of equipment used in malaria control were also tested during the period.

With a view to study whether, other factors remaining the same, a mere change in the distribution of the spray would make any difference in the prevalence of mosquitoes, one hut was sprayed with 1·25 per cent spray at 2 c.c. per sq. ft. uniformly, while another was sprayed with 2·5 per cent spray at 1 c.c. The latter gave a patchy spray due to the limited quantity though the dosage remained the same (25 mg. DDT per sq. ft. in both cases). Observation showed that patchy spraying afforded appreciably less protection ; spraying uniformly is essential to get the maximum benefits of residual spraying.

At Kallar (Nilgiris, S.I.) freedom from *A. fluviatilis* continued to be in evidence for over three months in the huts sprayed with different formulations (suspensions or emulsions) of DDT applied at the rate of 50 mgs. of DDT per sq. ft. and for about two months with gammexane suspension (P.520) applied at the rate of 15 mgs. gamma isomer per sq. ft. When, however, the quantity of gamma isomer per sq. ft. was doubled, the effect lasted for about three months.

Camphor-yielding *Ocimum* plants were successfully raised at Kallar (South India) for testing the repellent effect of the plant and its extracts on mosquitoes. Experiments conducted so far have not given any encouraging results.

RESEARCH PROGRAMME FOR 1950-51.

1. To study bionomics of different vector species particularly with regard to their nocturnal activity both in laboratory and in the field.
2. To study :—
   (*a*) The mode of action of insecticides in varying doses on different vector species.

(*b*) Systematic assaying of the effectiveness of different formulations of synthetic insecticides on the vector species.

(*c*) The types of formulations and the methods of application of sprays suitable for various types of surfaces met with in rural areas in India.

(*d*) Systematic testing of repellents, fumigants, larvicides and spraying equipment under field conditions in different parts of India.

3. To study the methods of control of *Culicines* by using the various formulations of DDT and gammexane, particularly as larvicides.

Dr. D. K. Viswanathan and Dr. B. Ananthaswamy Rao urged the necessity of continuing the enquiry. Dr. Viswanathan, however, suggested that it would be advisable to confine the investigations to only one specific aspect instead of various mentioned in the programme for the ensuing year. The Provinces could then duplicate the work from their own resources or with the financial assistance from the Indian Research Fund Association, if necessary. This would give additional assistance to the centre in the conduct of this work.

Both Colonel Chopra and Dr. D. N. Roy felt that since the Malaria Institute of India was an all-India research centre, it was wrong to curtail or limit the scope of the enquiry to one particular aspect only. They emphasized that the Malaria Institute of India was the only centre of its kind in the East and it was but proper that it must view its work from as wide an angle as was practicable.

Dr. A. C. Banerjea and Dr. D. K. Vishwanathan fully supported the proposed line of work but suggested that there should be collaboration amongst different research workers in order to enrich the present-day knowledge on the subject.

Colonel Jaswant Singh fully appreciated this point of view and felt that there could be no objection to observations being made on one or more vector species in different parts of India by different workers in close collaboration with one another. He undertook to send a circular to workers in India inviting suggestions and to incorporate them in the proposed programme, if feasible.

The inquiry was recommended unanimously.

2. *Inquiry on malaria under Dr. R. N. Chaudhuri, School of Tropical Medicine, Calcutta.*—Dr. R. N. Chaudhuri indicated at some length the work that had been done during the course of the year.

While determining comparative efficacy of paludrine, chloroquine and camoquin, an investigation with a single dose (0·5 g.), of each of these drugs was undertaken on 75 patients—36 *P. falciparum*, 35 *P. vivax*, 2 mixed (*P. falciparum* and *P. vivax*) and 2 *P. malariæ* infection, and the results obtained showed that asexual parasites disappeared from the peripheral blood within three days of the commencement of treatment in 80 per cent of paludrine and 100 per cent of chloroquine and camoquin treated cases. Fever was also controlled in the above ratios and it was observed that in bringing about a clinical cure all are effective, chloroquine being quick, paludrine slow and camoquin intermediate.

Among the different regimes of paludrine therapy further tried, a four-day course (0·6 g. on first day and 0·3 g. on the following three consecutive days) was found effective. It was economical and more convenient than the ten-day course. Eighteen *P. vivax* cases have so far been treated with combined paludrine 0·6 g. and plasmochin 0·2 g. daily for 10 days; two persons could not tolerate the full course, and one had clinical malaria after two months. Studies on plasma levels on paludrine in different doses were in progress.

A special study was made on a case of *P. falciparum* infection refractory to paludrine and on a fatal case with autopsy findings. Intramuscular paludrine was found to be more painful than intramuscular quinine, but the pain usually subsided in three days.

The experimental work on chemoprophylaxis of malaria in rural areas showed that there was a definite progressive decline in all the groups except the control. Persistence of infection in the affected individuals, a common feature in control group, was not noticed in any one of the treated groups. No untoward symptom was experienced in any group.

RESEARCH PROGRAMME FOR 1950-51.

1. To make a comparative study of the effects of paludrine in combination with (*i*) chloroquine and (*ii*) camoquin in indoor patients, with special reference to the immediate effect on fever and parasitæmia, toxicity, alteration in liver and gastric function and relapse rate.
2. To continue the study on combined therapy of paludrine and pamaquin or pentaquine on *vivax* malaria with special reference to the relapse rate.
3. To study what variations in urinary excretion of paludrine occur with different regimes of treatment and suppression with a view to find out any relation between the excretion of the drug and its anti-malarial activity.
4. To continue chemoprophylaxis of malaria in rural areas with paludrine, chloroquine and camoquin and, in addition, with quinine for comparison.

While Colonel Chopra fully supported the proposals, he asked Dr. Chaudhuri about the efficacy of paludrine in the treatment of acute cases of malaria, its toxic effects and the place of quinine alkaloids in the treatment of malaria; whether in view of the present-day economic situation in the Country cinchona alkaloids could still be used instead of synthetic drugs. Dr. Chaudhuri said that paludrine has some limitations in its use, that it was slower in action and hence would not be very useful in emergencies in M.T. infections. He preferred quinine or combination of quinine and paludrine in such cases. Quinine had also some limitations and may need to be used with pentaquine. Paludrine had little or no toxic effect. A short course of paludrine was much better than a long course. A total of 1·5 g. in 4 to 5 days with a leading dose of 0·6 g. had proved quite satisfactory. As regards Colonel Chopra's inquiry about stimulation of gametocytes by paludrine, Dr. Chaudhuri said that gametocytes persisted in the peripheral blood for 55 days.

At this the Chairman informed the Committee that while in this country, the viability of gametocytes was found to be only ten days, the workers at Horton had observed it to last for longer periods. Incidentally, he mentioned that workers in U.K. had demonstrated exo-erythrocytic forms in a piece of liver of a volunteer infected with *P. falciparum*.

Dr. Banerjea said that quinine being more anti-pyretic was more favoured by the general practitioners and the public than paludrine, although the latter had more parasiticidal value. He inquired what the chances of synthetic drugs completely replacing quinine were.

The Chairman drew the attention of the Committee to the report of the WHO Expert Committee on Malaria according to which quinine had a definite place in the treatment of malaria.

The inquiry was recommended unanimously.

3. *Inquiry on chemotherapy of malaria under Dr. K. Ganapathy at the Haffkine Institute, Bombay.*—Dr. Ganapathy gave a brief account of the work that had been done during the course of the year.

A large number of anti-malarial compounds were prepared to determine the action on the development of sporozoites, etc., in the infected mosquitoes and for suppressive activity test in *P. gallinaceum*. About 40 such compounds were synthesized and out of the ten tested for prophylactic activity in the mosquitoes, none proved effective. While testing two of the same for suppressive activity against *P. gallinaceum* infection in chicks one, however, gave good results. Another series of compounds numbering about 45 were prepared but not subjected to prophylactic tests.

Chloroquine of Haffkine Institute and two new quinoline compounds were also tested in dosages based on the known quinine equivalents of similar compounds. Chloroquine showed activity and one quinoline compound also appeared to be active in higher dosage.

RESEARCH PROGRAMME FOR 1950-51.

1. Synthesis of compounds likely to possess action on the sporozoites and exo-erythrocytic forms of plasmodia.

2. Evaluation of anti-malarial activity of new compounds against *P. gallinaceum* in the chick and in *Ædes ægypti*. Active compounds to be later tried against human plasmodia in *A. stephensi*.

While a number of compounds had been prepared, Dr. Ganapathy experienced some difficulty in their tests for want of chicks. The Chairman remarked that there were a number of compounds being prepared at the Malaria Institute of India as well and their tests would be undertaken from February onwards on return of the two officers of the Institute from studies abroad. The consensus of opinion was that more attention should be paid to the testing rather than the preparation of compounds and that proposals for the next year should be amended accordingly.

The Chairman drew the attention of the members to the directive given by the Scientific Advisory Board at their meeting held earlier in the morning namely that all proposals should be scrutinized from two points of view, viz. (*i*) whether the particular inquiry should be continued at all and (*ii*) whether it could be postponed.

The members generally felt that, while the work under this inquiry was useful, it was for consideration whether any economy could be effected. In view of the difficulties experienced in procuring the requisite chicks, Dr. Ganapathy agreed to reduce the proposed staff by one research assistant, two laboratory assistants and three laboratory attendants. With those amendments the enquiry was recommended unanimously.

4. *Inquiry on complement-fixation test in malaria for the assessment of anti-malarial drugs under Dr. J. C. Ray, Director, Indian Institute for Medical Research, Calcutta.*—The Chairman explained that this enquiry had been agreed to last year only for its intrinsic value and, in the absence of Dr. Ray, explained briefly what the plan of action was.

The inquiry began functioning on 28th May, 1949. For testing the different strains three monkeys were injected both intravenously and intramuscularly with blood containing respective parasites of *P. knowlesi*, *P. cynomolgi* and *P. inui*. On the third day when the blood slides were examined parasites were seen only in the monkey injected with *P. knowlesi*. The other two showed negative results. Attempts were made to increase the virulence of strains by (*i*) splenectomy or ligature of splenic artery and (*ii*) through mosquito infection.

For the preparation of anti-monkey hæmolysin three methods were tried by injection of normal saline suspensions of washed monkey r.b.c. in rabbits, viz. with a dose of (*i*) 2·5 c.c. of 5, 10, 15 and 20 per cent r.b.c., (*ii*) 1 c.c. to 5 c.c. of 50 per cent and (*iii*) 3 c.c. of 5 per cent, all injected intravenously. Hæmolysin titre obtained from serum from these methods was 1 in 50, less than 1 in 50 and 1 in 200, respectively. Anti-monkey hæmolysin is being prepared from 12 rabbits following the above methods with a slight change in dosage.

For want of virulent strains of *P. knowlesi* the work could not be carried out as outlined.

RESEARCH PROGRAMME FOR 1950-51.

1. To adopt methods for hæmolysis of r.b.c. for the extraction of *knowlesi* antigen.

2. To develop a dependable complement-fixation test for malaria with a standard antigen by a standardized technique.

3. To undertake work on the extraction and preparation of the antigens from the pre-erythrocytic schizönts of *P. cynomolgi* and their potency to be tested by complement-fixation reactions.

4. To extract merozoital antigen in order to test its

   (*i*) Potency for complement-fixation test in mammalian malaria.

   (*ii*) Capacity to induce merozoital immunity against human and monkey malaria.

The Committee were rather sceptic about the utility of the enquiry. The Chairman read out a letter from Dr. Ray clarifying the position about the proposed work. In this letter he had stated that Harris and Reidel after performing a large number of these tests in 1948 had found that, while the results were variable with sera from positive cases, they were highly satisfactory when negative sera and sera from patients suffering from some other diseases were used. The variable results with positive sera might be due to the potency of the antigen. Dulaney and Morrison by their special methods of extracts of antigen obtained a much stronger complement-fixation reaction. The test in malaria, as in its present form, might not serve significant purpose, yet it has value from the standpoint of diagnosis in those acute cases of *P. falciparum* infections where the parasites were conspicuous by their absence in the peripheral blood.

Evidence existed that there was an immune mechanism operative against the merozoites of the pre-erythrocytic forms but the immunological reactions against the latter forms differed from those produced by the sporozoites. Serological tests would facilitate the diagnosis of latent and relapsing forms of *vivax* infections if the immunological reactions produced by the trophozoites were also different from those against the exo-erythrocytic forms.

In his technical note, Dr. Ray had stated that the specific objectives of the proposed inquiry were to utilize the complement-fixation test in the diagnosis of malaria and in assaying of anti-malarial drugs and that he would like the Committee to give its whole-hearted support for the enquiry for these specific objectives.

The Committee did not recommend the continuation of the inquiry.

II. PROPOSAL FOR NEW INQUIRY.

Inquiry on the studies on the insecticidal and larvicidal properties of Indian flowers belonging to the N. O. Compositæ *under Dr. S. M. Ghosh, Assistant Professor of Entomology, School of Tropical Medicine, Calcutta.*—It was proposed to study the effect of the extracts of the flowers, Chamomilla, Chrysanthimum, Marigold and sun-flower on adult insects and their larvæ.

Dr. D. N. Roy pointed out that though a similar inquiry was negatived last year, he had restricted the scope of the inquiry proposed this year to a few flowers only.

Colonel Chopra was of the opinion that no useful purpose would be served by carrying out these investigations as most of the flowers had already been tested.

The Committee did not recommend this inquiry.

III. *To consider the proposals submitted by Dr. D. K. Vishwanathan regarding assessment of the validity of malaria morbidity statistics as recorded in out-patients departments by medical officers-in-charge of dispensaries in different levels of malaria endemicity.*—The Chairman explained that in another connection he had suggested to the Government of India that a small committee of experts be associated with the Registrar General to indicate how best to study the problems involved in the statistics of malaria morbidity. Dr. Viswanathan said that if such a committee was under contemplation by the Government he had no desire to press his proposals.

IV. *Work done on synthetic anti-malarials by Mr. P. R. Gupta, I. R. F. A. Research Fellow working under Dr. P. C. Guha, Professor of Organic Chemistry at the Indian Institute of Science, Bangalore.*—The Committee took note of the work done on synthetic anti-malarials by Mr. P. R. Gupta, I. R. F. A. Research Fellow working under Dr. P. C. Guha, Professor of Organic Chemistry at the Indian Institute of Science, Bangalore, and observed that it would be more useful to intensify the work on testing the compounds.

V. *Present status of anti-malarial drugs in the prophylaxis and treatment of malaria.*—The Committee took note of the present status of anti-malarial drugs in the prophylaxis and treatment of malaria as discussed in detail in the informal meetings on the 19th and 20th November, 1949 when most of the members of the Committee were present. At these meetings the report of the Third Session of the World Health Organization Expert Committee on Malaria was circulated and the members expressed their views on the various points contained in the report on malaria therapeutics. The general trend of the discussion favoured the conclusions arrived at by the WHO Expert Committee on Malaria, namely that quinine, mepacrine, chloroquine and paludrine when given in adequate dosage would nearly always terminate acute attacks of malaria; that in relapsing *vivax* malaria, concurrent treatment with quinine and an 8-aminoquinoline offered the best chance of radical cure and that as a suppressant, chloroquine and paludrine had proved effective in most countries.

It was, however, felt that in spite of the availability of the new and more potent synthetic anti-malaria drugs, the part which quinine would still play in the treatment of malaria in India, would continue to be of paramount importance, particularly because large quantities of cinchona products were obtainable from indigenous sources. It was also felt that for emergencies, the parenteral treatment with quinine had a definite value, as also in the treatment of relapses. Of the synthetic drugs the important ones from Indian point of view are mepacrine and paludrine. Chloroquine, pentaquine and isopentaquine are at present not available in the country. Chloroquine is the most expensive of the whole lot.

VI. *To recommend to the Scientific Advisory Board to initiate researches on those aspects of malariology which are not covered by the existing research programme in different institutions in India where malaria research is carried out.*—The Chairman explained that the object underlying this proposal was already being achieved through the circulars which were being sent by the Secretary, Indian Research Fund Association. Dr. Pandit was invited to give his views and he emphasized that the Advisory Committee, consisting as it did of experts available in the country, need not confine their discussions only to any schemes that may be put up by other workers, but could themselves give a lead by suggesting items of research which should be taken up by various workers.

The Committee recommended that a small sub-committee consisting of :—

Lieut.-Colonel Jaswant Singh (*Chairman*).
Dr. R. N. Chaudhuri
Dr. I. M. Puri
Dr. B. Ananthaswamy Rao
Dr. D. K. Viswanathan (*Secretary*).

be formed with the object of reviewing the researches already in progress and to recommend further research programmes which could be usefully carried out by the various organizations.

NUTRITION.

Nutrition researches under Dr. V. N. Patwardhan at the Nutrition Research Laboratories, Coonoor.

I. RESEARCHES.

EXCRETION OF THIAMINE AND RIBOFLAVIN BY INDIAN ADULT MALES.

The investigation reported last year has been extended further. A large number of observations have been recorded on 15 subjects under different dietary conditions. Excretion of thiamine and riboflavin were studied in 24-hour specimens and on fasting one-hour samples as well. The observations were made (*i*) when the subjects were living on their normal diets, (*ii*) when they were given self-selected diets of known composition, the diets being cooked and fed in the laboratory and (*iii*) when the subjects received uniform controlled diets for a definite period, also cooked and fed in the laboratory. Load tests with 1 mg. thiamine and 1 mg. riboflavin were carried out. The determinations of blood pyruvic acid and lactic acid and pyruvate : lactate (P : L) ratio in a few subjects were also undertaken. The results obtained and the conclusions drawn are given below in brief :—

(*a*). *Thiamine.*—(*i*) Daily excretions observed in 98 samples from 15 healthy subjects varied from 66 μg. to 1,200 μg., average being 339 μg. About 74 per cent of the observations fell between 100 μg. and 500 μg. The daily intake varied from 0·8 mg. to 1·3 mg. thiamine, only in one subject the intake was 2·13 mg. The percentage excreted ranged from 8·3 to 63·3 and bore no relation to the intake of thiamine.

(*ii*) Fasting one-hour excretions gave values from 3·4 μg. to 90·4 μg., the average being 15·5 μg. Nearly 80 per cent of the observations fell within 5 μg. to 20 μg. per hour.

(*iii*) When the excretion of thiamine was expressed per unit volume of urine, the values for 24-hour and fasting one-hour specimens were 0·03 μg. to ·49 μg. and 0·03 μg to 0·39 μg. per c.c., respectively. These results show that probably correct information concerning excretion could be obtained by the study of fasting one-hour specimens as well as by that of 24-hour specimen.

(*iv*) The proportion of the test dose returned within 24 hours varied from 3 to 55 per cent, the majority of subjects returned 6 to 20 per cent within 24 hours. The test dose return had no relation to the level of urinary-thiamine excretion.

(*v*) No correlation was observed between thiamine excretions and the number of non-fat calories in the diet.

(*vi*) The pyruvic acid content of blood in 15 subjects ranged from 0·5 to 1·28 mg./100 c.c. with an average value of 0·9 mg./100 c.c. Lactic acid content of blood in 8 subjects varied from 5·95 to 14·88 mg./100 c.c. with an average of 8·78 mg./100 c.c. The P : L ratio was 1 : 9. All these values fall within the reported normal range.

It can be concluded from the results of investigations described above that the daily allowance of thiamine as recommended by the Nutrition Advisory Committee, Indian Research Fund Association in 1944 appears to be adequate.

(*b*) *Riboflavin.*—(*i*) The range of daily excretion fell between 343 μg. and 4,401 μg. with an average of 1,243 μg. A majority of values were between 500 μg. and 1,300 μg. The daily intake of riboflavin varied from 0·76 mg. to 1·91 mg. The proportion of riboflavin excreted in urine had a range of 40 to 250 per cent; it bore no relation to the level of riboflavin intake.

(*ii*) Fasting one-hour excretion values ranged from 24 μg. to 143 μg.

(*iii*) When riboflavin excretion was expressed per unit volume the range of values was comparable between the 24-hour specimens and fasting one-hour excretions; the values for over 70 per cent observations varied between 0·3 and 0·7 μg./c.c. and 0·2 to 0·7 μg./c.c., respectively. There was, however, very poor correlation between the two sets of observations.

(*iv*) The test dose returns showed a wide range of variation, e.g. 4·4 to 85·3 per cent, and had no relation to the previous dietary intake or excretion levels of riboflavin.

(*v*) Urinary-riboflavin excretion was influenced by the protein and fat content of the diet and its carbohydrate : fat (C : F) ratio. At low C : F ratios, less riboflavin was excreted in urine, whereas the excretion was high at higher ratios. With an increase in protein content the riboflavin excretion decreased. Similar results were obtained with fat.

(*vi*) In a number of observations a very much larger amount of riboflavin was found excreted in urine than could be accounted for by the known intake. This suggests the possibility of microbial synthesis of the vitamin in the intestines. Much of the vitamin thus synthesized seems to be available to the human organism.

Work on thiamine and riboflavin metabolism is being continued further. Observations are now being made only on strictly controlled diets. Estimations of urinary and fæcal excretions are being carried out at known intake of these vitamins. The aim of the investigation is to determine the correlation between the fat, protein, carbohydrate content of the diet and the utilization of thiamine and riboflavin. Efforts will also be made to study the conditions under which biosynthesis of riboflavin can be influenced either way. In determining the dietary requirements of riboflavin it is essential to know the approximate contribution made by intestinal synthesis of this vitamin.

PYRIDOXINE AND FATTY ACID METABOLISM.

The experiments reported last year have yielded some interesting results. Young rats six to eight weeks in age were maintained on rations (*a*) pyridoxine-deficient, (*b*) fat-deficient and (*c*) fat-and-pyridoxine-deficient. A control group receiving both the missing factors was also kept. The deficiency symptoms in groups (*a*), (*b*) and (*c*) were dryness and scurfiness of the fur on hind paws, thorax and neck progressing to drying and thinning of hair especially over the face, round the eyes and on the venter. Scaliness of skin, dermatosis, erythema and swelling of hind paws were seen in more advanced stages. The animals were killed at this last stage and lipoids in serum, liver and kidney were analysed. It was observed that the appearance of deficiency symptoms was most rapid in fat and pyridoxine-deficient animals, less so in animals on fat-free diets and slowest in pyridoxine-deficient rats.

The lipoid analysis carried out in the advanced stages of deficiency revealed that the iodine values of liver and kidney fatty acids in fat-deficient and pyridoxine-deficient animals was lower than in the controls. The combined deficiency further lowered the unsaturation of fatty acids.

Another experiment was designed to study the curative effect of pyridoxine and fat singly or together in rats rendered deficient in both. It was observed that the external symptoms of the combined deficiency could be cured by exhibition of fat alone, whereas administration of pyridoxine alone had only partial effect. The results of lipoid analyses of the liver and kidney were, however, inconclusive.

The above experiments suggest a close relation between pyridoxine and fat metabolism. It is possible that the synthesis, intermediate metabolism and transport of fat may all be influenced by pyridoxine and essential fatty acids working together. Further experiments are in progress to study each of these effects.

PANTOTHENIC ACID DEFICIENCY AND ACETYLATION MECHANISM IN HUMAN SUBJECTS.

Gopalan (*Ind. Med. Gaz.*, **81**, p. 23, 1946) demonstrated the curative effect of panthenol in the 'burning-feet syndrome' and suggested that the latter condition was probably due to a deficiency of pantothenic acid. The diagnosis of the disease rests mainly on the interpretation of subjective symptoms described by the patient. It was desirable to have a means of objectively assessing the condition. Lipmann and co-workers (*Jour. Biol. Chem.*, **167**, p. 867, 1947) had shown that co-enzyme A which is concerned in the acetylation of sulphonamide and choline contained large amounts of pantothenic acid. Riggs and Hegsted (*Ibid.*, **172**, p. 544, 1948) demonstrated that acetylation of orally administered para-amino-benzoic acid (PAB) in rats depended upon their state of pantothenic acid nutrition. Attempts were, therefore, made to find out whether these observations could be utilized to develop a test for this deficiency in man. Oral doses of 100 mg. and 200 mg. PAB were administered to normals and patients of 'burning-feet syndrome' and the amount of acetylated PAB excreted in urine in 24 hours was determined. Repeat determinations were made on the same patients when distinct clinical improvement had set in after treatment with panthenol.

The results are given below :—

Dosage of PAB, mg.	Administration of riboflavin, mg.	Administration of panthenol, mg.	Number of normal cases.	Acetylation value in normal cases.	Number of cases of burning-feet syndrome.	Acetylation in cases of burning-feet syndrome.
100	...	...	11	89·5 to 93·8 Ave. 91·5	3	84·6 to 85·6 Ave. 84·9
200	...	...	8	89·7 to 92·4 Ave. 91·0	4	77·7 to 79·5 Ave. 78·9
100	125	...	1	92	1	84·2
100	...	350	1	90·7	1	87·6
200	...	350 to 750	2	91·6	6	86·9 to 90·8 Ave. 88·3

It will be clear from the above Table that the capacity to acetylate PAB was depressed in the patients and that the defect was more marked at 200 mg. than at 100 mg. level of dosage. It is suggested, therefore, that this test could be applied to detect cases of burning-feet syndrome.

The subject, however, requires further study. It was found that in malaria the acetylation mechanism seemed to suffer. It is also obvious that in any condition where medication with sulpha drugs has been carried out the acetylation test cannot be undertaken unless a sufficient period is allowed to elapse after the last administration of the drug in order to free the body of residual sulpha drug.

STUDIES ON VITAMIN D.

(*a*) *Vitamin D content of butter and ghee.*—Vitamin D has been assayed by the 'line test' technique in three samples of butter and three samples of ghee. The non-saponifiable fractions were used for the assay. Values ranging from 600 to 1,850 I.U. per g. butter were obtained. Untreated whole ghee gave values of 1,040 to 1,540 I.U. /per 100 g. It was observed that values obtained on the non-saponifiable fraction were considerably lower than those obtained with untreated samples.

The vitamin D content of one sample of egg-yolk as determined on the non-saponifiable fraction was 500 I.U. /100 g. of egg-yolk.

All the values given above are considerably higher than those reported in the literature. While further assays are obviously needed, it is suggested that a higher incidence of sunlight all round the year might be responsible for the high values observed in the samples derived from the Indian animals.

(*b*) *Vitamin D action.*—It was reported last year that vitamin D as such had no local action on rachitic bone calcifying *in vitro*. In continuation of last year's work further experiments were carried out by injecting large doses of vitamin D intravenously in sheep and studying the calcification of rachitic slices in serum obtained from blood drawn before and after the administration of vitamin D. In some experiments evidence of better calcification was obtained in serum from blood drawn 24 to 48 hours after vitamin D injection. The results, however, were not uniform and there were some inherent difficulties due to (*a*) loss of capacity of the serum to promote calcification on standing at room temperature or at 0°C. and (*b*) loss of calcifying power of bone slices kept for 24 hours or more. This mode of attack was, therefore, temporarily abandoned in favour of another one which has yielded interesting results.

It is well known that even after a single massive dose there is a certain time lag before the action of vitamin D becomes manifest. The available descriptions of histological changes in the epiphyses refer to those which follow the first appearance of a calcifying line in the provisional zone of calcification in the hypertrophic cartilage. The experiments described below provide evidence to believe that the changes which take place still earlier are not yet properly understood.

Young rats were fed rachitogenic diets for fifteen days. To some 4,000 I.U. vitamin D in oil was given orally, to others only a drop of vegetable oil. After 24, 48, 72 and 96 hours both groups were killed, tibiæ were removed and slices made. Control slices were immediately fixed in 10 per cent formal saline.

The remainder were incubated in a calcifying solution of composition Ca : inorg. P : ester P of 10 : 4 : 1 for 21 hours at 37°C., after which they were washed and stained with silver nitrate.

The following observations were made in a number of such experiments :—

1. The formalin-fixed control slices of all animals not receiving vitamin D showed complete absence of calcification as was to be expected. Similar slices from rats to whom a single dose of 4,000 I.U. was administered did not give evidence of any calcification till a period of 96 hours had passed after ingestion of vitamin D.
2. On incubation with calcifying solutions of identical composition, (*a*) the bone slices of rachitic rats showed minimal or no calcification, whereas (*b*) rachitic rats which had received a single dose of vitamin D (4,000 I. U) showed calcification commencing after 24 hours; the extent and intensity of calcification increased progressively in the 48-, 72- and 96-hour periods. It is to be noted that in none of these bone was there any evidence of calcification having commenced *in vivo*.

These results indicate that it is not merely the increase in Ca and inorganic P of the blood under the influence of vitamin D that determines the laying down of the bone salt, but that rachitic bone undergoes some as yet ill-understood change, probably in the organic matrix, which has to be completed before calcification *in vivo* can begin. It is probable that a study of these preparatory changes may reveal the secret of vitamin D action. The biochemical studies are at present in progress and during the next year attempts will be made to correlate the biochemical and structural changes which precede calcification *in vivo*.

STUDIES IN PROTEIN METABOLISM :

INFLUENCE OF DIETARY PROTEIN ON NITROGEN BALANCES.

Several observers in India have commented upon the low level of urinary-nitrogen excretion observed in the normal healthy Indian adults. The reasons advanced in explanation were that (*a*) the protein intake of Indians was low and (*b*) the low digestibility of vegetable proteins which formed the major portion of protein in Indian diets rendered only a fraction of the ingested protein available to the body. Both these explanations were seldom put to actual proof. It is interesting to note that Wilson and Mukherji (*Ind. Jour. Med. Res.*, **23**, p. 483, 1935) and Basu and Basak (*Ibid*, **27**, p. 115, 1939) have recorded comparatively low urinary N values even on nitrogen intakes exceeding 10 g. per day. In their observations and those of others as well, there were fairly large N balances which remained unaccounted for. It was obvious that further work was needed. In some preliminary experiments undertaken in Bombay in 1945-46 it was observed that at constant protein intake the amount of urinary N depended upon the type of protein mixture given in the diet. This was of course well known, for on such differences are based the methods of finding out the biological value of proteins. On the other hand results obtained in the present study were entirely different from those expected, and hence a further study of the problem was undertaken at Coonoor.

Four adult volunteer subjects were selected for study. Their normal diets were surveyed and urine N in 24-hour collections was estimated. They were then placed on diets prepared from weighed foodstuffs which did not materially differ from their own. These diets were prepared and fed in the laboratory under supervision to two of the subjects. The other two, being research worker volunteers themselves, were allowed to prepare and consume the diets in their houses but separately from other members of the household. A preliminary period of seven to ten days was allowed, during the latter part of which urine N was determined daily. When the latter reached a steady state, fæces and urine were both collected for two or three periods of two days each and N determined in both. After this the protein component of the diet was modified with the incorporation of meat, milk (reconstituted from whole-milk powder) or eggs in place of rice, wheat or pulses. The caloric content was adjusted by incorporation of sago in the diet. Each article of the diet was analysed for its total N content.

The first period was always that corresponding to predominantly vegetarian diets (basal diet). In later periods animal protein was increased till it formed 50 per cent or over of the total protein in an iso-caloric diet. This was done in one or two stages. Finally, the subject was returned on the basal diet. Determinations of N in urine and fæces were repeated during each of these periods after the steady state had been reached. The findings are summarized below :—

1. The urine nitrogen excreted per 24 hours was distinctly higher on diets containing a higher proportion of animal protein than on predominantly vegetarian diets. The fæcal nitrogen, on the other hand, remained unaltered or showed insignificant variation with the change in the diets.
2. As a result of changes mentioned in 1, the nitrogen retention was higher in vegetable-protein periods than in animal-protein periods. Such changes in N retention could be repeatedly demonstrated by alternating vegetable- and animal-protein diet periods.
3. The apparent digestibility of protein during the two periods was practically of the same order.
4. The results lead to the conclusion that the low urine N observed in Indians cannot be ascribed either to (*a*) low protein intake or (*b*) low digestibility of vegetable proteins.

Although the daily retention of a certain amount of nitrogen is to be expected the large N balance encountered in this investigation and reported by other Indian workers must be considered unusual. The fact that the same subject when placed on animal proteins shows a decreased retention certainly requires some explanation. Throughout the duration of the experiment weights of the subjects were either steady or showed an increase not greater than 2 lb. in any individual case. The retained N was, therefore, not utilized to any large extent for the building up of new tissue. The climate of Coonoor and the type of work on which the subjects were engaged eliminate the possibility of dermal losses through sweating.

Any suggestions advanced to explain N retention must take into account the above-mentioned differences observed on vegetable- and animal-protein diets. The possibilities which could be investigated in the first instance are the influence on N retentions of (*a*) the non-protein nitrogen in the foodstuffs and (*b*) the amino-acid composition of the proteins. Further work on this interesting problem is in progress.

INFLUENCE OF NUTRITIONAL FACTORS IN LIVER DISEASE.

A. *The effect of poor rice diets.*—The experiments described last year were continued and observations were made after 12, 15 and 21 months after feeding the poor rice diets. No further biochemical changes of any significance could be noted at these intervals. The histological examination of livers of rats killed at 15 and 21 months showed pathological changes. The sections of livers of three out of four rats killed at 21 months showed evidence of fatty degeneration In none of the livers, however, was there any evidence of proliferation of connective tissue. The remaining four rats in this series were killed after 21 months. Microscopically the liver appeared normal. On histological examination, evidence of fatty degeneration was found in two livers. In the other two marked rarefaction of cytoplasm of liver-cells was seen. There was no evidence of connective tissue hyperplasia in any of the sections.

Such experiments are time-consuming and tedious and at the end give results which are not always clear-cut. In the present set of experiments it has been demonstrated with reasonable certainty that if rats are fed poor diets for prolonged periods certain pathological changes can be expected to occur in the parenchyma of the organ. The diet used in this investigation was partially deficient in several food factors, hence the ætiological role of any one or all of them is not quite clear. It is also not certain whether these early pathological changes could be considered the precursors of hepatic cirrhosis. So far as the present observations are concerned it seems necessary to draw attention to the possible role of methionine. Before this is discussed it would, however, be desirable to give in brief the main observations made on the livers during the whole course of the experiment :—

(*i*) The liver weights of rats on poor rice diets (PRD) in relation to body-weights were higher than those of stock-diet (SD) animals at two months after the beginning of the experiment. Towards the end, however, the difference had narrowed down considerably.

(*ii*) At two months the total nitrogen content of PRD-rat livers was distinctly lower than that of SD-animals. During fifteen months of the experiment, the differences had become negligible owing to greater increase in total N of PRD-rat livers.

(*iii*) To begin with, the crude-fatty-acid (CFA) content of PRD-rat livers was higher than that of SD-animal livers. After four months, CFA in PRD-rat livers reached a maximum value of over 10 per cent and later gradually declined only to show a mild increase after 12 months. During this same period the CFA of livers of SD-animals remained more or less constant at normal levels.

(*iv*) The methionine content of livers of PRD-rats was lower throughout the experiment than in livers of SD-animals. In this the liver methionine reflected the dietary methionine levels. Cystine content of livers in different groups did not differ appreciably at any particular period.

(*v*) The pathological changes in the livers of PRD-animals were seen first at 15 months and also in a group killed at 21 months. These consisted of necrosis, fatty degeneration and marked rarefaction of cytoplasm of the liver-cells. There were no signs of connective tissue hyperplasia.

B. *The role of methionine.*—The above findings suggested that probably methionine deficiency was the cause of the biochemical and histological changes found in the livers of PRD-animals. It is to be noted that the diets were low both in methionine and choline. Choline is required to exert lipotropic activity. When this nutrient is deficient its deficiency can be made good by methionine if the latter is available. As the supply of both was limited in the experiments reported above methionine was required during the period of active growth for building up of new tissue-protein as well as to make up the deficiency of choline. The results showed that the demand for growth was more important than for lipotropic function and hence methionine was not fully available for the latter. When the growth ceased, more of methionine was diverted for lipotropic activity and the fat content of livers of PRD-animals did then decrease. This hypothesis was put to test by supplementing the PRD with methionine and choline respectively and studying their effects on liver-fat after four months of feeding to a fresh set of animals. The results are interesting and are given below :—

Diets.	Results of liver analyses at four months per 100 g. of fresh liver.	
	CFA g.	Cystine g.
Poor rice diet (PRD)	10·52	0·49
PRD + choline/1 mg./rat/day ...	3·74	0·40
PRD + dl-methionine 4 mg./rat/day ...	3·26	0·43

C. *Role of methionine in liver injury caused by carbon tetrachloride.*—From the above experiments it was clear that methionine was one of the important factors in certain changes observed in the liver on an unsatisfactory diet and hence a guess may be permitted that due to the continued low intake it was probably the cause of liver injury seen in the final periods of the experiment. That this was possibly correct can be seen by the results of methionine supplementation in toxic injuries of liver reported by other workers both in clinical as well as experimental fields. An attempt was made to find out the influence of toxic injuries on the liver methionine content and the effect methionine had when diet was supplemented with it. For this experiment the technique described by Radhakrishna Rao (*Ind. Jour. Med. Res.*, **23**, p. 1007, 1935) was followed. Carbon tetrachloride 0·1 c.c. was injected subcutaneously twice a week in adult rats kept on (*a*) poor rice diet (PRD), (*b*) PRD *plus* dl-methionine 8 mg./rat/day and (*c*) stock diet (SD). Controls on PRD and SD were also kept at the same time.

The results showed that (*i*) all rats in group (*a*) died on receiving 12 to 21 injections, (*ii*) all rats in group (*b*) survived, and (*iv*) all rats in group (*c*) survived. This left little doubt that in toxic injury there were extra demands on methionine. That the dietary supplement of methionine was effective is shown by the survival of all animals in this group and the higher methionine content of their livers. In this connection it is interesting to note that the animals who survived did show on histological examination greater damage to the livers than those which died on poor rice diet. It is not possible to comment on this aspect at this stage.

EXPERIMENTAL LATHYRISM.

It must be admitted that the investigation on lathyrism made little progress during the last year. Two dogs from the second experimental batch showed an early sign of the disease in that stiffness of hind limbs and abnormality of gait were discernible after a few months of feeding *Lathyrus* diet. Unfortunately, the condition has not progressed any further in spite of continuous feeding with the *Lathyrus* diet for a year, and is milder still than in the dog from the 1947 experiment.

A detailed investigation of the blood constituents did not reveal any appreciable differences between the control and experimental animals.

Repeated attempts were also made to induce toxic effects in guinea-pigs such as those described by Bhagvat (*Ind. Jour. Med. Res.*, **34,** p. 299, 1946) but they did not meet with success.

One possible explanation which can be offered for the observed variability of effect is that probably *Lathyrus sativus* itself must be non-toxic, the disease lathyrism being due to presence of some toxic species of *Lathyrus* in the marketed crop. During the last three years the material for experiments at the Nutrition Research Laboratories had to be obtained from four or five different sources, and it may well be that the extent of contamination varied according to the locality in which the crop was grown. Confirmatory evidence has been forthcoming from other sources. Information was received that in the United Provinces and Bihar, *L. sphericus* (a toxic variety) is found mixed with the *L. sativus* crop. On the other hand reports from Central Provinces point to an absence of such contamination.

Some circumstantial evidence also exists to connect the lathyrism outbreaks with the consumption of the possibly contaminated *L. sativus.* In view of these reports and observations it is felt desirable to re-plan the whole experiment along the following lines : (*i*) To investigate the extent of contamination of *Lathyrus sativus* crops with toxic species of *Lathyrus* wherever the crop is grown. (*ii*) To collect further information on the incidence of lathyrism and its association with contamination of the *Lathyrus* crop. (*iii*) A field investigation on lathyrism in accordance with the recommendations of the Lathyrism Sub-Committee, Indian Research Fund Association. (*iv*) Laboratory experiments with pure-bred strains of *Lathyrus sativus* and *Lathyrus sphericus* (a toxic variety) on suitable animals with pure and mixed grains.

The assistance of the Indian Council of Agricultural Research is being sought on the agricultural aspects of the problem. Field work will be undertaken in collaboration with the health authorities of a province in which lathyrism is endemic. The laboratory experiments will be undertaken when purebred strains become available.

CLINICAL INVESTIGATIONS.

The Nutrition Clinic has continued to show progress by attracting a greater variety of cases of nutritional disorders. During the last year, 207 cases have been examined and treated at the Clinic. The distribution of cases according to the deficiency and syndromes is given below :—

Particulars.	In-patients.	Out-patients.
Vitamin A deficiency	8	50
Riboflavin deficiency	1	14
Thiamine deficiency	—	3
Vitamin C deficiency	—	2
Phrynoderma	—	48
Mixed deficiencies :		
Vitamin A + riboflavin ...	2	2
Vitamin A + riboflavin + thiamine	—	3
Vitamin A + vitamin C ...	—	1
Thiamine + riboflavin ...	—	5
Pellagra	—	1
Burning-feet syndrome ...	2	11
Deficiency œdema	2	6
Malignant malnutrition ...	18	—
General malnutrition including marasmus	3	10
Anæmia :		
(*a*) Iron deficiency	—	1
(*b*) Nutritional macrocytic ...	3	1
Total ...	39	158

Ætiology of phrynoderma.—During the course of the last year a controlled study on the treatment of phrynoderma was rendered possible through the co-operation of a local orphanage. In this institution 48 girls out of a total of 80 were found to suffer from the condition in varying degrees of severity. The diets of these children had been surveyed and it was decided not to recommend any change in the diet during the course of the investigation. The patients were divided into a number of groups containing almost equal number of mild, moderate and moderately severe cases. The analyses of serum-lipoids of all these patients were done before treatment. It was observed that although there were no differences in the total lipoids and sterols in blood between this group and girls of comparable age groups living on the same diets but free from phrynoderma, the iodine value of serum fatty acids in the former group was significantly lower than that observed in non-phrynoderma subjects.

The patients were then treated for six weeks on daily doses of (*i*) marmite ½ ounce, (*ii*) gingelly (*Sesamum indicum*) oil ¼ ounce to 1 ounce, (*iii*) gingelly oil 1 ounce + marmite ½ ounce. The clinical response was as follows :—

(*i*) *Marmite.*—There was no change in the condition of the skin or the follicles during the six weeks of treatment.

(*ii*) *Gingelly oil.*—The improvement in general condition of the skin was evident within 10 to 14 days. In mild cases the follicles had disappeared by the sixth week, in moderate and severe cases they did not completely disappear, but were very much flatter and smoother. No appreciable difference in improvement was noticeable among the groups on three doses of gingelly oil—¼ ounce, ½ ounce and 1 ounce—tried in this experiment.

(*iii*) The addition of marmite to gingelly oil did not influence the response to any appreciable extent.

One other group which was kept under observation without treatment did not show any change in the condition at the end of six weeks. Still another group which received skimmed milk powder two ounce per day also showed no appreciable change.

Analyses of serum-lipoids of all these children after six weeks of treatment showed that wherever clinical response had been good, there was a significant rise in the iodine value of serum fatty acids. Where there was no response the latter had remained almost stationary.

Four aspects require emphasis in the interpretation of results :—

1. The very low fat content of the diet. In 35 out of 48 girls the fat intake on an average was 8·4 g., in the remaining 13 it was 36 g. per day.
2. The average vitamin A values of the diet for these two groups were 3,920 and 8,600 I.U., respectively.
3. In the treatment an oil was used which was the source of essential fatty acids. Gingelly oil contains linoleic acid to the extent of 40 per cent of its fatty-acid composition.
4. A favourable clinical response was accompanied by a significant increase in the degree of unsaturation of serum fatty acids.

All these facts put together point to the ætiological role of essential fatty acids in the causation of phrynoderma. There is, however, one aspect which requires further investigation. It was found that some patients had phrynoderma in spite of the daily intake of 36 g. fat on an average. Two possibilities suggest themselves, either the supply of essential fatty acids through the dietary fat was inadequate or that the utilization of an adequate supply of this nutrient was influenced adversely by some as yet unknown factor(s). It is intended to clarify the position in the coming year.

Malignant malnutrition.—In the last year's report this condition was described under ' Infantile Pellagra '. For reasons given there and from subsequent experience with a fresh batch of cases it appears that malignant malnutrition would be a better term.

During the last year 18 cases have been investigated. They were children 15 to 42 months in age and in all, the presenting symptoms were steatorrhœa, and œdema with dry skin, with or without branny desquamation and in nine cases typical dermal lesions described by Ramalingaswami, Menon and Venkatachalam (*Ind. Physician*, **7**, p. 229, 1948). Angular stomatitis was present in six patients and xerosis with Bitot's spots in three. In only four patients liver was palpable. Blood picture showed the presence of anæmia of dimorphic or hypochromic type. Five of the patients showed on fæces examination the presence of roundworm ova.

There is little doubt now that the disease is identical in most respects with a similar condition called Kwashiorkor in South and East Africa. Although the ætiology of the disease is unknown it is now almost certain that chronic malnutrition of the infants in post-weaning period with special reference to low protein intake has an important role in the development of the syndrome. It has been suggested that continued protein deficiency after weaning may cause atrophic changes in the pancreas and decreased secretory activity of the gland which in turn would influence the digestion and absorption of nutrients from the intestines thus enhancing the effects of malnutrition. The effects on liver are, according to Davies (*Lancet*, **i**, p. 317, 1948), secondary to pancreatic insufficiency.

There is no doubt that protein deficiency existed in the patients investigated at Coonoor for their dietetic history revealed that after weaning, the children were fed mostly with rice kanji, other foods being given only infrequently. It was, therefore, decided to obtain evidence for pancreatic insufficiency and deficient liver function in these patients by determinations of serum lipase and serum esterase in addition to the usual clinical investigations on blood, urine and fæces. The details of investigations, treatment and results are given below :—

Treatment and results.

Treatment.	Number treated.	Cured.		Died.	
		Number.	Remarks.	Number.	Remarks.
Skim milk	10	9	Number of days under treatment : 17 to 43 days average 31.	1	Died on 4th day without any improvement.
Skim milk *plus* dried de-fatted pancreas.	4	1	Cured in 21 days.	3	
Skim milk *plus* lipocaic preparation.	1	1	Cured in 41 days.	—	
Skim milk *plus* vitamin A concentrate.	3	3	Average range 20 to 60 days.	—	

Results of biochemical investigations before and after treatment.

	Before.		After.	
	Number.		Number.	
Total plasma protein g. %				
Range ...	12	3·35 to 4·30	8	6·10 to 8·7
Average ...		3·70		7·43
Plasma-esterase units / 100 c.c.				
Range ...	12	30 to 205	8	100 to 180
Average ...		88·3		139·8
Plasma lipase units / 100 c.c.				
Range ...	4	20 to 40	4	100 to 160
Average ...		31·3		122·5

The number of patients is admittedly small, and generalizations will be hazardous. It would be in order, however, to point out some significant facts emerging from these preliminary studies:—

(*a*) The poor dietary history, the low plasma proteins and the cure obtained with skim milk alone shows that the condition may be considered to originate in a protein deficiency. The possibility of other concurrent deficiencies cannot, however, be excluded.

(*b*) The values for plasma lipase point to either (*i*) pancreatic insufficiency or (*ii*) defective absorption due to any other cause or both. The low esterase values are suggestive of deficient liver function.

(*c*) The feeding of de-fatted de-hydrated pancreas or lipocaic preparation have not yielded encouraging results so far.

(*d*) Mortality in the present series has been comparatively small, only 4 out of 18 cases terminated fatally, whereas in the earlier series 9 out of 10 patients died under treatment.

The points of interest which require further investigation are (*i*) the effect of protein or protein hydrolysates alone on the progress and cure of the syndrome, (*ii*) intestinal intubation studies to distinguish pancreatic insufficiency from defective absorption in the intestines, (*iii*) determination of serum esterase, lipase and trypsin and (*iv*) liver biopsies.

It has not been possible to obtain material for post-mortem examination. Attempts will also be made to secure such material for detailed study whenever possible.

Another important line of investigation which it is proposed to follow is to observe in a stable population of one or two villages, the development of the syndrome in children. It is hoped that by this means it will be possible to obtain detailed information about the ætiology of the disease and to detect cases in their early states. A follow-up of the children recovered from the disease will yield information about the after effects which the disease may have on the patients during adolescence.

II. FIELD WORK.

1. Routine nutrition surveys.

School children in and around Coonoor have been examined for the evaluation of nutritional status. The *pro forma* adopted by the Nutrition Advisory Committee in December 1948 has been utilized for observing and recording the results of the examination. In the physical examination of children two additional items of information were considered desirable, viz. the presence or absence of palpable liver and spleen. Appropriate entries were, therefore, added to the schedule under 'alimentary system'. In all 741 children have thus far been examined; hæmoglobin was also estimated in each child by Wong's method. The results of the survey are being analysed.

Dr. A. Gilroy, Director of the Ross Institute of Tropical Hygiene, Assam was interested in the evaluation of nutrition of children on the tea estates of Assam. He has carried out the routine examination of 450 children according to the Nutrition Advisory Committee Schedule and has sent the relevant forms duly filled to the Nutrition Research Laboratories for analysis and comment. The material is at present under investigation.

2. Nutrition surveys supported by laboratory investigations.

An intensive survey of this type was undertaken in an orphanage in Coonoor. The orphanage had 16 children between 1 and 4 years, 36 children between 5 and 11 years and 23 children between 12 and 20 years. In addition to the routine examination carried out in accordance with the Nutrition Advisory Committee Schedule, the examinations of blood, urine and fæces were done in all subjects. The determinations of basal metabolic rate (BMR), urinary-thiamine and riboflavin excretions, blood-lactic and pyruvic acids, and dark-adaptation were undertaken in selected subjects.

The following are the conclusions of the study :—

(*a*) The heights and weights of the children were found markedly lower than those of South Indian children of comparable age.

(*b*) Only 7 out of 75 children could be classified as good, the remainder being fair to very poor.

(*c*) Examination of the fæces showed wide prevalence of helminth infestation, the most common being *ascariasis* frequently associated with *trichuriasis*. Tape-worm ova were found in three and *ankylostome* ova in one.

(*d*) Hæmatological investigations showed the presence of iron-deficiency in 35 children.

(*e*) BMR of 10 subjects—graded very poor—gave an average value of 43·0 cal. /m^2 /hour as against 40·4 cal. /m^2 /hour for six 'good' children of comparable age. The numbers were too small to enable any conclusions being drawn.

(*f*) Data for riboflavin and thiamine excretions and pyruvic and lactic acid in blood are given below. It is to be noted that riboflavin excretion in the 'very poor' grade was nearly one-third of that recorded with the 'good' grade children.

Particulars.	"Very poor" children.		"Good" children.	
	Number.	Average.	Number.	Average.
1. Thiamine in urine				
(*a*) 24 hours	18	371 μg.	7	386 μg.
(*b*) Fasting 1 hour	16	25 ,,	6	32 ,,
2. Riboflavin in urine				
(*a*) 24 hours	12	351 ,,	7	946 ,,
(*b*) Fasting 1 hour	11	31 ,,	6	105 ,,
3. Pyruvic acid in blood	18	0·96 mg./100 ml.	7	1·16 mg./100 ml.
4. Lactic acid in blood	18	11·51 mg./100 ml.	7	14·01 mg./100 ml.
5. P : L ratio		1 : 11·9		1 : 12·0

Circumcorneal pigmentation.—During the nutrition surveys mentioned above the incidence of circumcorneal pigmentation was encountered in the children being examined. Dhurandhar and Boman Behram (*Ind. Jour. Med. Res.*, **27**, p. 735, 1940) first described this condition in the inmates of an industrial school in Bombay and reported an incidence of 89·4 per cent, whereas in a paying day school they found it to be 26·8 per cent. Gilroy (1940) in a personal communication reported finding similar pigmentation in association with xerosis among the children of tea estate population in Assam.

In the Nilgiris an incidence of 80·56 per cent has been found among a total of 746 children examined thus far. The pigment occurs as rings or segments with clearly defined margins around the cornea at the sclerocorneal junction or in the sclera at a distance of about 0·5 mm. from the cornea; the colour varies from light-brown to dark-brown. In most cases the pigment is in continuity with the cornea, but in some it is distinct from it on the medial and lateral sides. When the pigmented ring surrounds the cornea, it appears to be separated and drawn away from the former in the equatorial region.

It appears, therefore, that this type of pigmentation of the conjunctiva is more common than was till now realized. Its association with xerosis in the present series was not so marked as reported by Dhurandhar and Boman Behram and also noted by Gilroy in Bombay and Assam, respectively. Among the boys examined, 68 showed evidence of xerosis of varying degrees and 61 of these, i.e. 89·7 per cent had pigmented conjunctiva as described above. The percentage incidence of pigmentation in xerosis-free subjects was 79·6, whether this difference is significant is too early to say. Further investigation is obviously necessary before the ætiology of pigmentation can be understood. The above findings have been mentioned here with a view to drawing the attention of workers interested in the field of nutrition.

III. EDUCATION AND ADVISORY WORK.

Inquiries from the public and the Governmental agencies for information and advice have been on an increase. On some occasions analytical work was called for, but such work was only undertaken on request on behalf of Governments. General educational activity consisted in writing of articles in semi-scientific and popular journals, lectures at conferences and broadcast talks. The Nutrition Museum is being gradually expanded.

Health Bulletin No. 23 "The Nutritive Value of Indian Foods and the Planning of Satisfactory Diets" has been completely revised. Several portions of the bulletin have been re-written and the Tables enlarged so as to contain larger number of entries and additional information on individual foodstuffs. Synonyms in the more important Indian languages have been incorporated. The revised edition is now in press.

Health Bulletin No. 30 "Food and Diet (written for children)" was also revised during the last year. The revised edition of this bulletin also is now in press.

The annual course in nutrition was held from 14th March, 1949 to 30th June, 1949.

2. Nutrition research unit under Professor B. C. Guha at the University College of Science & Technology, Calcutta.

A. STUDIES IN THE BIOSYNTHESIS OF ASCORBIC ACID.

(*i*) Study of —

(*a*) The effect of chloretone on the formation of ascorbic acid in germinating seeds of *Phaseolus radiatus*, either alone or in presence of thiamine and riboflavin.

(*b*) The effect of thiamine or riboflavin or both on the formation of ascorbic acid without any chloretone being added.

(*c*) The effect of irradiation on the chloretonized and vitaminized seeds.

(*ii*) Study of the effect of ultra-violet irradiation and sunlight on the biosynthesis of ascorbic acid in rats.

(*iii*) Study of the oxidizing enzyme or substance in the tissues and urine of rats, either normal or narcotized.

(*i*) *The effect of chloretone and other factors on the formation of ascorbic acid in germinating seeds of* Phaseolus radiatus.

It has been shown by biological assay with guinea-pigs, reported previously, that the indophenol-reducing substance formed in germinating *Phaseolus radiatus* on exposure to sunlight or ultra-violet light (3,000-4,000A°) is very probably vitamin C. Having found this, it was considered of interest to investigate how this synthesis of the vitamin in germinating seeds would be affected by chloretone, thiamine and riboflavin, as these agents have been shown previously to affect the biosynthesis of the vitamin in rats.

It has been observed that chloretone has also a stimulating effect on the formation of ascorbic acid during the germination of the seeds of

Phaseolus radiatus. This effect is not appreciably reinforced by exposure to ultra-violet light. The addition, however, of thiamine or riboflavin or both appears to have a slight further stimulating effect on the formation of vitamin C by the chloretonized seeds. Thiamine or riboflavin or both have also by themselves a fairly marked stimulating effect on the synthesis of ascorbic acid by the seeds without any chloretone being added. Further irradiation of these vitaminized seeds has a slightly more stimulating effect on the biosynthesis of ascorbic acid. It would thus appear that chloretone, thiamine and riboflavin play some part in the synthesis of ascorbic acid in these germinating seeds, as they do in rats. This seems to indicate some similarity between the mechanisms of the biosynthesis of vitamin C in the animal and the plant organisms studied :—

Experimental.—The mature seeds (*Phaseolus radiatus*), as obtained in a fairly fresh condition from the market were first cleaned, thoroughly washed and air-dried. A known quantity of these seeds (1 g. each) was then allowed to germinate in clean and dry Petri-dishes, containing known volume (10 c.c.) of either distilled water or chloretone solution (0·025 to 0·1 per cent) either alone or in presence of 10 mg. per cent solution of thiamine and riboflavin each. The effect of the addition of 10 mg. per cent solution of thiamine or riboflavin or both, to the distilled water-medium containing the seeds was also studied. It has been observed that chloretone, in higher concentrations above 0·05 per cent tends to inhibit the germination. 0·3 per cent solution of chloretone inhibits the germination of *Phaseolus radiatus* almost totally.

After 24 or 48 hours germination, as the case was, the seedlings were transferred together with the germinating solutions into a clean glass-mortar, 2 c.c. of 10 per cent trichloro-acetic acid solution were added, the mixture was ground to a fine suspension and finally centrifuged. The ascorbic acid content of the seedlings, germinated under different conditions was determined by titration against 2 : 6 di-chlorophenol-indophenol.

(ii) *The effect of ultra-violet light and sunlight on the biosynthesis of ascorbic acid in rats.*

It has been reported before that sun rays and ultra-violet rays of wave-lengths 3,000-4,000A°, as transmitted by the Wood's filter, are effective in stimulating the formation of ascorbic acid during the germination of seeds. Whether sun rays and ultra-violet rays can also accelerate the biosynthesis of ascorbic acid in rat was next studied. No significant change in the biosynthesis of ascorbic acid in rats was noticed due to exposure to sun rays or ultra-violet irradiation.

Experimental.—Thirty six rats weighing 60 g. to 92 g. were taken and divided equally into four groups. Rats were separately placed inside metabolism cages, kept in a dark-room and maintained on a stock diet which was kept also in darkness. The rats of the first group were kept in darkness during the whole experimental period. The second group kept in darkness was exposed to sun rays varying from 15 minutes to 6 hours a day during the twenty-one days of the experimental period. The third and fourth groups, also kept in darkness, were exposed to ultra-violet light. The third group was only exposed to ultra-violet light of wave-lengths 3,000-4,000A°, using Wood's filter. The fourth group was exposed to ultra-violet light without using any filter. Before exposing the rat to ultra-violet light, or sun rays, the hair of the rat were removed by means of fine scissors. All the rats of the third group from different cages were placed inside one metabolism cage and were exposed to ultra-violet light in such a way that the distance between the animal and lamp was 8″ and the duration of exposure varied from 10 minutes to 180 minutes per day. In the animals of the fourth group the distance between the lamp and the rats was 28″ and the time of exposure varied from 10 to 100 minutes. During the experimental period of twenty-one days, the urine of all the rats was examined for vitamin C contents. After the experimental period of twenty-one days, rats of all the groups were sacrificed and the vitamin C contents of different tissues were estimated with the indophenol indicator.

(*iii*) *Study of the oxidizing enzyme or substance in the tissues of rat, either normal or narcotized.*

Oxidation of ascorbic acid in plants by a special enzyme ascorbic acid oxidase has been demonstrated and its existence in plants has also been established. Its occurrence in the animal is, however, questionable. It may be possible that the presence of some oxidizing enzyme or substance, which oxidizes the vitamin C in rats in course of its normal synthesis, may be responsible for less excretion of vitamin C in the urine of normal rats. In the case of narcotized rat, however, the action of this oxidizing enzyme or substance might be inhibited and thereby the loss of vitamin C might be less and hence more of free vitamin C is excreted in the urine of narcotized rats. In other words, the increased excretion of ascorbic acid by the narcotized rat might rather be due to the inhibition of the natural oxidation of ascorbic acid by the narcotic in the tissues than to the increased synthesis of ascorbic acid induced by the narcotic. An examination of the urine and liver and kidney tissue of normal and chloretonized rats, with added ascorbic acid, shows that the results are comparable and that the increased excretion of ascorbic acid by chloretonized rats is not due to any ascorbic acid oxidizing system being inhibited in the chloretonized animal.

B. FATTY ACID OXIDATION BY THE GUINEA-PIG AND THE RAT.

(*i*) *Fatty acid oxidation by the liver-tissue of the scorbutic guinea-pig.*

It was reported last year that the rate of oxidation of sodium caprylate by the tissue slices from the liver of guinea-pigs was not affected by scorbutic condition of the animals. The work was continued on the following lines : Harrison (*Biochem. Jour.*, **27**, p. 1501, 1933) and Quastel and Wheatley (*Ibid.*, **28**, p. 1014, 1934) reported that ascorbic acid accelerates the respiration of scorbutic liver *in vitro* in presence of crotonate and butyrate. So the effect of added ascorbic acid on the rate of oxidation of sodium caprylate by slices of liver from scorbutic guinea-pigs was measured in Warburg's apparatus by the method of Jowett and Quastel (*Biochem. Jour.*, **29**, p. 2143, 1935). 0·2 c.c. of a solution containing 2 mg. to 5 mg. of ascorbic acid in normal saline was brought to pH 7·4 by addition of $\frac{N}{10}$ NaOH and was added to the reaction medium. The results show that the rate of oxidation of caprylic acid by slices of liver from scorbutic guinea-pigs is definitely increased by the addition of ascorbic acid. This increase, however, may either be due to the oxidation of the added ascorbic acid or due to the increased oxygen uptake initiated in the liver slices by the addition of ascorbic acid. Harrison (*loc. cit.*) and Quastel and Wheatley (*loc. cit.*) appear to be inclined to the latter view.

In order to test which view is correct, it is proposed to examine the rate of disappearance of the added ascorbic acid from the glycerophosphate—Ringer medium in presence of the liver tissues of the scorbutic guinea-pigs by the titrimetric technique and compare this rate with the rate of oxygen uptake observed before. Incidentally, experiments of this nature have been carried out with normal guinea-pig liver tissue and it has been found that of 2·5 mg. ascorbic acid added 0·2 mg. disappeared in one hour, showing that although ascorbic acid is largely protected from destruction by the normal liver-tissue, as has been observed by many workers, there is definitely a small proportion of the vitamin which does disappear even in presence of the liver-tissue. So it appears conceivable that the increased oxidation observed in the experiments of Harrison, and Quastel and Wheatley in scorbutic liver-tissue might be due not so much to the oxidation of some fatty acid induced by the added ascorbic acid but to the oxidation of the added ascorbic acid itself.

In this connection it may be mentioned that Spellberg (*Proc. Soc. Exper. Biol. Med.*, **41**, p. 570, 1939) reported the development of fatty liver on scorbutic diet. Longenecker *et al.* (*Arch. Biochem.*, **5**, pp. 137-51, 1944), however, found no difference in the amount and nature of liver-fat of the normal and scorbutic guinea-pigs. In order to throw further light on this question the amount of fat in the liver of guinea-pigs was determined in three groups of animals consisting of six guinea-pigs each and receiving the following diets :—

Group I	..	Scorbutic diet *ad lib.* +5 mg. of ascorbic acid/animal/day.
Group II	..	Scorbutic diet *ad lib.*
Group III	..	Scorbutic diet +5 mg. ascorbic acid/animal/day ; given restricted amounts of diet as voluntarily consumed by animals of group II according to the paired feeding technique.

The results show that the variation in the amount of liver-fat of the three groups of animals was not significant, the mean percentages of the three groups being 3·73, 3·50, and 3·19, respectively. The iodine numbers of the total fat of liver of normal (group I) and paired (group III) groups were practically equal but it was higher in the scorbutic group by about 20 per cent. This would seem to confirm the findings of Longenecker *et al.* (*loc. cit.*) and it would appear that fat metabolism is not materially affected by the scorbutic condition.

(*ii*) *Fatty acid oxidation by thiamine deficient rats.*

The rate of oxidation of sodium caprylate by the slice of liver as well as the amount and iodine value of liver-fat were determined in the following three groups of rats : The animals of the first group received thiamine-free diet *ad lib.*, the animals of the second group received thiamine-free diet paired with the animals of the first group together with 0·5 mg. of thiamine per animal per day and the third group received thiamine-free diet *ad lib.* together with 0·5 mg. of thiamine per animal per day.

It appears from the result that the increase in the oxygen uptake of the liver tissue in the presence of sodium caprylate is the least in the thiamine-deficient group. This increase is higher in the other two groups being very nearly of the same order in both. The percentage of fat in liver is less in the thiamine-deficient group than in the other two groups. The iodine number of the liver-fat is the highest in the thiamine-deficient group and least in the paired group, the value in the normal group being intermediate. It is thus surprising to notice that, although unsaturation of fat is the greatest in the thiamine-deficient rats, the oxygen uptake is the least which may be due to the deficiency in thiamine which is necessary for the oxidation of carborhydrate degradation products.

C. STUDIES ON FOOD-IRON.

Work on food-iron has been carried out this year according to the following plan :—

(*i*) Hæmopoietic effect of iron of lentil (*Lens esculenta*) and lecitho-vitellin in nutritionally anæmic rats.

(*ii*) Effect of calcium as calcium carbonate on the absorption of iron of ferric phytate and ferric chloride by rats.

(*iii*) Effect of vegetable protein (glutenin, gliadin, pea's globulin) on the hæmopoietic effect of ferric chloride.

(*iv*) Absorption and excretion of iron by rats.

(*i*) *Hæmopoietic effect of iron of lentil* (Lens esculenta) *and lecitho-vitellin.*

The hæmopoietic effect of a few iron-bearing foodstuffs on nutritionally anæmic rats was investigated last year. In continuation of that work investigation on the hæmopoietic effect of lentil (*Lens esculenta*) and lecitho-vitellin (an iron-bearing phospho-protein prepared from ducks' egg-yolk) was undertaken.

Rats were made anæmic according to the technique of Elvehjem *et al.* (*Jour. Biol. Chem.*, **93**, p.189, 1931). When the rats were 6 to 7 weeks old, their hæmoglobin level generally came down to 40 to 60 per cent. The rats were divided into two groups with respect to sex, body-weight and hæmoglobin level. The rats used were from three different litters. While the first group which was given lentil consisted of five rats, the second group which was given lecitho-vitellin consisted of four rats. The diets of the two groups are described below :—

Group I ..	Each rat received boiled lentil, amount of lentil supplied per rat per day being 2·2 g. containing 0·3 mg. of iron. Supplement of copper 0·03 mg. as 0·1 c.c. of copper-sulphate solution was given per rat per day orally. When the rats had taken their quota of lentil each day, they were then given milk *ad lib.*
Group II ..	Each rat received 0·71 g. of lecitho-vitellin daily supplying 0·3 mg. of iron. The lecitho-vitellin was suspended in a 3 c.c. portion of cow's milk to which was also added 0·03 mg. of copper in

0·1 c.c. of copper-sulphate solution. After the rats had taken lecitho-vitellin supplement, they were given cow's milk *ad lib.*

Each rat of both the groups was given two drops of 'Adexolin' weekly. The period of experiment lasted 30 days. Hæmoglobin was measured by Sahil's hæmatometer once in 10 days with the blood obtained from a cut at the tip of the tail of the rat. At the end of the experimental feeding period rats were sacrificed and their livers and spleens were analysed for total iron content.

The results obtained indicate that with lentil, rise in hæmoglobin during the thirty days of experimental feeding period is 10 per cent, while the corresponding figure with lecitho-vitellin protein is 38 per cent. Thus, the iron of lentil in hæmoglobin regeneration behaves more or less like that in rice, wheat and ferric phytate. The effect obtained with lecitho-vitellin is more pronounced than that obtained with ferric chloride.

The order of figures for the iron content of livers and spleens of rats of different groups is in agreement with the order in the values for hæmoglobin rise. The amount of iron stored in livers and spleens of rats given lecitho-vitellin is greater than the corresponding amounts obtained with rats given lentil.

(*ii*) *Effect of calcium on the absorption of iron of ferric phytate and ferric chloride by rats.*

There seems to be some difference of opinion as to whether calcium promotes or inhibits iron absorption. Sterman (*Dep. Bull. Exper. Stn., U. S. Dept. Agric.*, No. 185, 1907), Orten (*Amer. Jour. Dis. Child.*, **54**, p. 252, 1937 and Day and Stein (*Jour. Nutr.*, **16**, p. 525, 1938) considered that additional calcium tended to promote iron utilization. Kleitzein (*Jour. Nutr.*, **19**, p. 187, 1940), on the other hand, found that the addition of calcium salts to the diet of anæmic rats interfered with their ability to absorb iron. While studying the relation of the dietary calcium and phosphorus ratio to iron assimilation in nutritionally anæmic rats Elvehjem (*Jour. Lab. Clin. Med.*, **25**, p. 464, 1940) observed that iron utilization was most efficient at the lowest Ca : P ratio and was progressively reduced with higher ratios. The observations of Tompsett (*Biochem. Jour.*, **34**, p. 961, 1940) also indicate that iron is absorbed more easily on a low calcium diet.

Working with ferric phytate as the source of iron it has been observed that iron of ferric phytate is but poorly absorbed from the intestine of rats, on the other hand the iron of ferric chloride is appreciably absorbed. In order to see the effect of calcium on the absorption of iron from these two sources the following work was undertaken :—

Six litters of rats after their weaning period were kept on milk diet. When the rats were of 60 g. to 65 g. body-weight, they were divided into four groups, taking care of even distribution of body-weight, sex and litter mates. While groups I and III consisted each of six rats, groups II and IV had five rats each. The groups were given diets as described below :—

While rats of groups I and III were given Klim whole-milk powder to which three per cent calcium carbonate (made iron-free) was incorporated, rats of groups II and IV were given Klim milk powder only. Rats of groups I and II were given a supplement of 0·25 mg. iron as ferric chloride per rat per day. Rats of groups II and IV were given 0·25 mg. of iron as ferric phytate. Each rat was given daily 0·03 mg. copper as copper sulphate and two drops of Adexolin per week. Feeding period was for four weeks. During the first week of the experiment fæces of the rats of different groups were collected for iron estimation. At the end of the experimental feeding period rats were killed and after removing the entire intestinal tract, the body-iron was estimated. Iron was estimated in livers and spleens were estimated for iron separately.

The results show that the addition of calcium carbonate under the conditions studied interferes with and hence lowers the absorption of iron from the gastro-intestinal tract whatever may be the source of food-iron.

The values of body-iron, liver-iron and spleen-iron also indicate that addition of calcium carbonate to the diet lowers the amount of stored iron of the body.

(*iii*) *Effect of vegetable proteins on the hæmopoietic effect of iron of ferric chloride.*

In the last year's report, the effects of three animal proteins, egg-albumin, egg-yolk and casein, were studied on the hæmopoietic effect of iron of rice. This year, three vegetable proteins, gliadin and glutenin from wheat and globulin from peas, have been investigated as to their effect on the hæmoglobin regenerating value of iron of rice. The work, however, is still in progress.

(*iv*) *Absorption and excretion of iron by rats.*

From a study of the absorption and excretion of iron by normal persons, McCance and Widdowson (*Lancet*, **2**, p. 680, 1937) have suggested that the capacity of the body to excrete iron may be very much less than has generally been supposed. There is no dispute over the excretion of urinary iron which is agreed by all to be very small and constant in amount (Lanyar *et al.*, *Physiol. Chem.*, **217**, p. 160, 1933 ; McCance and Widdowson, *Quart. Jour. Med.*, *n.s.* **6**, p. 277, 1937). The quantity of iron lost through the gastro-intestinal tract with the fæces has also been reported to be small (Widdowson and McCance *Jour. Physiol.*, **94**, p. 148, 1938 ; Widdowson and McCance, *Biochem. Jour.*, **31**, p. 2029, 1937). They say that, "Obviously negligible and non-regulatory excretion with great storage capacity of the body for iron must be coupled with limited and to some extent controlled absorption". It is accordingly suggested by the authors that absorption of iron is conditioned by the relative concentration of free ions in the lumen of the gut and in the cells. At the physiological level of intake only a very small fraction of ingested iron is absorbed.

Absorption of iron by rats.—The hypothesis of McCance and Widdowson (*loc. cit.*) on the metabolism of iron is silent on the question of the availability of food iron. According to the authors, at the physiological levels of intake of iron, only a small fraction of ingested iron is absorbed. Even if we accept the above statement, the question remains whether the small fraction of ingested iron which is being absorbed at the physiological level of intake is independent of the ionizability of the food-iron ingested. In order to investigate the above problem two sets of experiments were carried out. In the first place, two groups of rats of body-weight 42 g. to 85 g. reared on milk diet after the weaning period were fed two different sources of food-iron with different ionizabilities. While the rats of one group were given ferric ammon-citrate with 100 per cent ionizability, the rats of the other group were given hæmatin with 100 per cent non-ionizable iron as the source of iron. At the end of the experimental feeding period the rats were killed and body-iron, liver-iron and spleen-iron were estimated.

In another set of experiments, adult rats of body-weights varying between 154 g. and 230 g. reared on laboratory stock ration were chosen as experimental animals. The rats were divided into two groups and were given supplements as described before *plus* milk *ad lib*. At the end of the experiments, the rats were killed and body-iron estimated.

The results indicate that a considerable amount of iron from ferric ammon-citrate and a comparatively very low amount of iron from hæmatin were absorbed in the first set of experiments. In the second set of experiments, only a negligible amount of iron was absorbed whether it came from ferric ammon-citrate or from hæmatin.

On the basis of the above work it appears probable that young rats which are growing can absorb iron; the ionizability of the iron from the source is of importance under these conditions. Adult rats, on the other hand, can absorb but little iron whatever may be its ionizability.

Excretion of iron by rats.—Generally, the excretion of iron through the kidney is neglected. There are, however, references according to which urine contains definite but small amounts of iron. It has also been reported that excretion of iron through the kidney is enhanced under various conditions (McCance and Widdowson, *Quart. Jour. Med.*, **6**, p. 277, 1937; Widdowson and McCance, *Jour. Physiol.*, **94**, p. 148, 1938; Hahn and Whipple, *Jour. Exper. Med.*, **70**, p. 443, 1939).

Coming to the excretion of iron through the intestinal tract it has been reported by McCance and Widdowson that the human intestinal tract cannot excrete iron. Working with dogs Hahn and Whipple reported that the body controls its iron storage by absorption or lack of it rather than by its capacity to eliminate it.

In order to test the excretory power of rats to eliminate iron either by the kidney or by the intestinal tract, the following experiments were planned:—

Four groups of normal adult rats kept on laboratory stock ration were taken. All the rats during the experimental feeding period were given only cow's milk *ad lib*. The experimental feeding period consisted of two halves. The first half lasted seven days only when the second half started. During the second half different groups were given different supplements or treatments. Group I was given cow's milk alone as before. Group II was given, in addition, a supplement of iron as ferric ammon-citrate. Group III was given injections of iron, while group IV was given injection of phenyl-hydrazine solution to produce an hæmolytic anæmia. During the whole experimental feeding period, fæces and urines were analysed for iron on alternate days.

It has been observed that rats excrete a definite and more or less fixed amount of iron with the urine. The amount excreted with the urine is found to be unaffected by the amount of iron taken in with the food or by the injection of phenyl-hydrazine. However, injection of iron in the third group increased the amount of iron in the urine, this increase was only a minute fraction of the amount of iron injected.

Coming to the question of excretion of iron with the fæces it has been observed that neither the injection of iron nor the anæmic condition produced by the injection of phenyl-hydrazine has any effect on the amount of iron excreted with the fæces. Feeding of a heavy dose of iron increases the amount of iron in the fæces correspondingly. The major fraction of this iron in the fæces comes from the unabsorbed iron of the supplement.

All these observations lead to the conclusion that the capacity of rats to eliminate iron either by the kidney or by the gastro-intestinal tract is limited. If any extra amount of iron could be forced into the system by injection, it would not be eliminated but would remain stored in the system. If we feed rats a high dose of iron, almost the whole of it is excreted with the fæces unabsorbed. The destruction of red blood corpuscles and hæmoglobin by phenyl-hydrazine injection which increased the 'free' iron in the serum does not enhance the excretion of iron either by the kidney or by the intestinal tract. However, it should be stressed that the body does excrete iron through the kidney and through the gastro-intestinal tract—the amount excreted through the gastro-intestinal tract being much larger than the amount excreted with the urine.

3. Nutrition research unit under Dr. R. G. Chitre at the Seth G. S. Medical College, Bombay.

I. FAT METABOLISM.

(a) *Absorption of fat from human intestine.*

As stated in the last year's report, the effect of inorganic phosphate on the absorption of fat in the presence of a carbohydrate was further studied. The sugars dealt with were glucose, fructose, lactose and sucrose, and the investigations were carried out on human beings under normal physiological conditions. The standard meal consisted of 1 oz. butter, 2 oz. bread and a cup of tea. The experimental subjects were divided into four groups, each group being subjected to a series of three experiments as follows :—

(*i*) With 1 oz. butter. (*ii*) With 1 oz. butter and 1 oz. carbohydrate. (*iii*) With 1 oz. butter, 1 oz. carbohydrate and 2 g. of Na_2HPO_4.

The experimental subject was made to fast for at least 10 to 12 hours before he or she was given the test-meal and blood samples were drawn out from the finger-tips just before the test-meal and afterwards at hourly intervals for the next five hours. The rate of absorption of fat was studied by using the Frazer's chylomicron technique.

Some results are given below :—

TABLE I.

Chylomicron counts showing the effect of inorganic phosphate on the absorption of fat from human intestine in the presence of 1 oz. of carbohydrate.

Time, hours.	1 oz. butter.	1 oz. butter + 1 oz. glucose.	1 oz. butter + 1 oz. glucose +2 oz. Na_2HPO_4	1 oz. butter.	1 oz. butter +1 g. fructose.	1 oz. butter +1 oz. fructose + 2 g. Na_2HPO_4
0	3·0	1·5	2·3	2·5	2·6	1·4
1	5·2	5·6	12·5	3·4	2·3	1·9
2	35·2	7·8	4·4	19·4	7·3	14·8
3	19·7	25·6	41·4	32·8	26·8	3·3
4	9·4	38·6	32·1	10·4	12·2	35·0
5	6·4	20·0	18·4	4·9	12·5	14·4

TABLE II.

Time, hours.	1 oz. butter.	1 oz. butter +1 oz. lactose.	1 oz. butter +1 oz. lactose +2 g. Na_2HPO_4	1 oz. butter.	1 oz. butter +	1 oz. butter + + 2 g. Na_2HPO_4
0	1·7	3·9	4·1	7·8	3·0	3·6
1	7·7	6·3	2·3	4·3	4·3	3·6
2	38·1	4·9	27·4	6·2	6·6	3·8
3	41·4	13·4	21·6	9·6	17·7	3·6
4	3·9	14·9	31·5	2·3	47·7	13·6
5	7·4	18·6	21·7	2·7	34·7	19·4

When butter alone was ingested the majority of cases gave the maximum count of fat globules between the 2nd and 3rd hours. When glucose, lactose and sucrose were given along with butter, many cases showed a delay of an hour or two in the maximum count. Feeding of Na_2HPO_4 together with butter and carbohydrate, however, restored the original rate in some cases, but in others it was not significantly effective. No such delay was found in the case of fructose-fed groups.

It is highly probable that the delay in attaining the maximum count in the glucose- and the dissaccharide-fed groups may be due to a competitive demand for the phosphate radical between fat and glucose, whereas no such competition exists between fat and fructose which are supposed to be absorbed partly by pure diffusion and partly by slow change-over to glucose. Hence the absorption of butter is not affected when it is fed simultaneously with fructose.

The question whether 2 g. of inorganic phosphate fed simultaneously with butter and a carbohydrate can restore the original rate of absorption of the former is still undecided.

(*b*) *Comparative effect of hydrogenated groundnut oil, groundnut oil and butter on the composition of body-fat of albino rats.*

The comparative nutritive values of vanaspati, groundnut oil and butter with respect to growth, reproduction and clinical symptoms had been reported last year. The work was further continued with a view to investigate the effect of fat feeding on the composition of body-fat of animals of a subsequent generation. The tissues (blood, liver, brain, muscle, kidney and spleen) were analysed for total fatty acids, cholesterol, phospholipids and iodine value.

Results.—Cholesterol : In case of animals kept on low fat diet, the value of cholesterol content of the various tissues of the vanaspati group was higher than that of butter group, while for animals maintained on high fat diet, the cholesterol content was lower for the former group than for the latter.

Total fatty acids : Butter groups gave higher results for total fatty acids than vanaspati and groundnut oil groups. This was true for both, low fat as well as high fat fed animals.

Phospholipids : The phospholipid values for the three groups fell in the order of vanaspati, butter, groundnut oil.

Iodine value : No consistent difference was found between the iodine value of the total fatty acids of the animals fed with different types of fat at different levels.

II. STUDIES IN EXPERIMENTAL RICKETS.

Mode of action of vitamin D.

Experiments have been undertaken to study (*i*) the nature of the serum-alkaline-phosphatase with a view to find out whether the bone is its source and (*ii*) the mode of action of vitamin D using the respiration technique of Warburg.

The serum-alkaline-phosphatase is increased not only in the bone diseases but also in other diseases in which the bone is not involved at all, such as, obstructive jaundice, hyper-thyroidism, hepatic cirrhosis. Attempts are, therefore, made to get some experimental evidence as to whether the bone is at all the source of the enzyme. Some arguments that it is not so have been detailed in the last year's report. The problem is, however, being further investigated from another angle.

Blood filtrate (trichloroacetic acid treated) is being used as substrate (a source of organic phosphorus) so as to have, as far as possible, a physiological substrate. On this the activities of phosphatases both in serum and in bone extract are being studied with a view to finding out whether there is any difference in the nature of bone and serum-phosphatases. In another set of experiments, the enzyme is separated from the serum-proteins and its activity is then studied on the blood-filtrate, in order to get an idea regarding the nature of interference of the proteins with the phosphatase activity. The work is continuing and no definite conclusions at this stage can be given.

Work is also being undertaken to study, by means of the respiratory technique of Warburg, the mode of action of vitamin D. Slices of bones from normal, rachitic and hyper-vitaminotic rats are incubated in the Warburg apparatus in the sera of normal, rachitic and hyper-vitaminotic rats and their oxygen consumption compared. The technique of the study of the mode of action of vitamin D has been developed in this laboratory so as not to duplicate the work that is being carried out at Coonoor where the technique of *in vitro* calcification is being developed for the purpose.

III. ANALYSES OF FOODSTUFFS.

(*a*) *Amino-acid content of various cereals and pulses.*

Data regarding the amino-acid contents of the Indian cereals and pulses being so far not available, work was undertaken to carry out the routine analyses of these foodstuffs as regards their amino-acid contents. The essential amino-acids determined were methionine, cystine, histidine and tryptophane in various samples of rice, wheat and pulses such as lentil, mung, chavli, pavta, etc.

(*b*) *Vitamin content of some cereals and pulses.*

Some cereals and pulses commonly forming part of the diet of the people in this province have been analysed for their vitamin contents. So far nicotinic acid and carotene only have been estimated in them and the results obtained have shown that barley is the best source of nicotinic acid, while green gram is of carotene. The work regarding the other vitamins particularly vitamins B_1 and B_2 complex has just been undertaken.

(*c*) *Effect of storage on the thiamine and riboflavin content of rice and wheat samples.*

Samples of rice and wheat were received from Government of Bombay in the year 1944. They have since been stored under ordinary conditions. Rice was stored with its husk on and thymol was used as a general preservative. All the samples were kept at room temperature in well-stoppered bottles. On undertaking estimation of thiamine and riboflavin in these samples, not one showed trace of either of these vitamins, thereby indicating that the conditions under which they have been stored were in no way conducive to the retention of these vitamins. Changes in nicotinic acid content are being studied.

(*d*) *Analyses of the foodstuffs sent by the Food Department of the Government of India.*

Some work has been undertaken at the instance of Food Department of the Government of India concerning analyses of some foodstuffs, viz., groundnut cake, wheat flour, tapioca and sweet potato flour, in respect of carotene, nicotinic acid, thiamine and riboflavin. Samples are being received from time to time and data submitted to the Food Department.

IV. THE PHYSIOLOGICAL AVAILABILITY OF VITAMINS OF B COMPLEX.

In the last year's report, mention has been made of the studies on the 'physiological availability' of nicotinic acid by using the technique of 'in *vivo*-digestion' of the foodstuffs and comparing the results with those of the 'in *vitro*-degestion'. It has now been decided to apply the same technique to the estimation of other water-soluble vitamins, viz. thiamine and riboflavin in several foodstuffs and the work has been started.

V. NUTRITIVE VALUE OF MILK AND CURDS.

During the year under review, the problem was further studied from the point of view of changes in vitamin contents during curdling for varying lengths of time by pure cultures of *L. casei* and *L. arabinosus*. Vitamins studied were nicotinic acid, thiamine and riboflavin. The mean results are shown in Table III :—

TABLE III.

Changes in vitamin contents of milk during curdling by L. casei *and* L. arabinosus.

Hours.	Nicotinic acid in μg./g.		Thiamine in μg./g.		Riboflavin in μg./g.	
	L. casei	*L. arabinosus*	*L. casei*	*L. arabinosus*	*L. casei*	*L. arabinosus*
0	0·65	0·65	0·23	0·23	0·68	0·68
24	0·54	0·57	0·19	0·21	0·74	0·72
48	0·48	0·54	0·24	0·22	0·74	0·84
72	0·45	0·50	0·23	0·25	0·85	0·89

The results indicated that nicotinic acid decreased, while riboflavin increased with the duration of curdling. Thiamine content was, however, unaffected.

4. Inquiry on the nutritive value of soya-bean milk and nutrition research unit under Mr. B. N. Banerjee and Dr. S. S. De at the Indian Institute of Science, Bangalore.

I. STUDIES ON THE NUTRITIVE VALUE OF SOYA-BEAN MILK AND CURD.

This work was carried out in co-operation with Dr. V. N. Patwardhan, Director, Nutrition Research Laboratories, Coonoor, and Dr. V. Subrahmanyan, Planning Officer, Central Food Technological Research Institute, Mysore.

(i) *Feeding experiments with soya-bean milk.*—The Institution feeding experiments with soya-bean milk that had been started in June 1948 were continued and completed by the end of 1948. The observation on heights and weights of the subjects at the beginning and at the end of the experimental period was statistically analysed. The following general conclusions were drawn :—

The difference in height and weight of the subjects in groups A and B receiving cow's milk and soya-milk as a supplement respectively was not statistically different. The number of subjects in the control group receiving no supplement was not large enough to permit an evaluation of the comparative nutritive value of soya-milk and cow's milk on a percentage basis. The result of the feeding experiments during 1947 and 1948 are submitted in a separate report.

(ii) *Feeding experiments with soya-bean curd* (Bangalore School Feeding Scheme).— The feeding experiment that had been started in Malleswaram Boys' Middle School, Bangalore, on 300 boys was conducted for five months. The experiment included three groups of subjects under the following treatments :—

GROUP A (100 subjects) .. 8 oz. cooked rice + 8 oz. separated milk curd.

GROUP B (100 subjects) .. 8 oz. cooked rice + 8 oz. soya-bean curd.

GROUP C (100 subjects) .. Control—(no supplement).

Increases in heights and weights of the children in the three groups were recorded at monthly intervals. The data at the beginning and at the conclusion of the feeding period were statistically analysed. The various results are summarized below :—

Distribution of the age of the children in the treatment.

Age group, years.	Number of subjects.		
	Group A.	Group B.	Group C.
12 ..	26	25	26
11 ..	33	34	34
10 ..	27	27	27
9 ..	8	8	8
8 ..	5	5	5
Total ..	99	99	100

Mean growth in weight and height.

Age group, years.	Weight in oz.		
	Group A.	Group B.	Group C.
12 ..	48·36	43·36	30·15
11 ..	40·13	37·07	24·12
10 ..	41·63	38·88	18·37
9 and 8 ..	28·00	25·33	23·53

Age group, years.	Height in inches.		
	Group A.	Group B.	Group C.
12 ...	0·66	0·70	0·68
11 ...	0·70	0·78	0·54
10 ...	0·73	0·70	0·47
9 and 8 ...	0·67	0·77	0·80

Mean differences in growth between treatments.

	Weight.			Height.		
	Mean diff.	t	5% t	Mean diff.	t	5% t
$X_a—X_b$ ..	3·66	0·85	1·96	0·0360	0·73	1·93
$X_a—X_c$ ..	15·48	4·26	1·96	0·1413	3·12	1·93
$X_b—X_c$ ..	11·82	2·93	1·96	0·1773	3·63	1·93

The above tables show that with respect to increase in weight and height, each one of the treatments A and B is showing significantly greater increase than control group C. Between groups A and B significant difference is, however, not noticeable.

The above mid-day school feeding experiment showed that soya-bean curd has a nutritive value comparable with that of separated milk curd when they are fed to children along with rice as a supplement.

II. Effect of feeding coffee extracts to rats receiving a poor South Indian diet.

Two groups of young rats (12 in each group) were taken. Group I received the usual South Indian rice diet. The other group received along with the rice diet, supplements of coffee extracts. Each rat received the water extract from 1 g. of coffee powder per day. The feeding experiments were continued for seven weeks.

It was found that in the early stage of growth (first two weeks) the feeding of coffee produced a retarding effect. At the end of seven weeks, however, there was not much difference in the total growth of the rats in the two groups. The rats receiving coffee extracts consumed on the average more food than rats receiving no supplement. For equivalent amounts of food intake, the average increase in weight was 27·8 g. and 31·9 g. (for groups I and II respectively). No harmful effect of feeding the coffee was noticeable.

III. Studies on the diet survey of the labour-class population in Bangalore.

The object of this investigation was to ascertain the quantity of foodstuffs consumed by the labour-class people and to gain a knowledge as to their nutritional status by carrying out an analysis of the food consumed for protein, fat, minerals and vitamins. It was also hoped that it would be possible to suggest cheap and effective supplements to make diet adequate and balanced nutritionally.

Information about the various classes of foodstuffs consumed by a group of 100 families drawn from the sweepers' colony and labour colony of the Bangalore Municipality and belonging to an income group of Rs. 50 to Rs. 100 per month was obtained. A representative group of six families was chosen among them and the food consumed by them was weighed daily by house-to-house visit for 15 days. An aliquot of the foodstuffs consumed daily was collected. At the end of the collection period, the pooled aliquots for each family were analysed.

The chief cereals consumed were rice, wheat flour, maize flour, cholam flour and ragi. Tur dhal, Bengal gram were the chief pulses. The vegetables included brinjals, onions, amaranth, potato and tomato. Small amounts of pork and buffalo flesh were consumed. Gingelly oil and groundnut oil and milk were consumed in small amounts. On an average the daily consumption of various classes of foodstuffs in g. per consumption unit was as follows :—

Dry weight of food.	Cereals.	Pulses.	Vegetables and fruits.	Fats and oils.	Flesh food.	Milk and milk products.
607 g.	471 g.	34 g.	103 g.	8 g.	35 g.	65 g.

By analysis, the amount of the various proximate principles and vitamins and minerals consumed per consumption unit per day were found to be as follows :—

Protein ..	60·3 g.	Thiamine ..	1,320 µg. (Micrograms).
Fat ..	21·0 g.	Riboflavin ..	690 Micrograms.
Calcium ..	0·884 g.	Nicotinic acid ..	45 mg.
Phosphorus ..	1·753 g.	Ascorbic acid ..	39 mg.
Calories ..	2,165	Carotene ..	170 Micrograms.

The diet is deficient in pulses, oils and fats and sources of animal protein. The calorific deficiency is about 15 per cent, cereals contributing most of the calories. The intake of good quality animal protein is small. Intake of fat is very low. Since ragi, a cereal rich in calcium, was consumed, the intake of calcium is adequately met. The diet is deficient in all the vitamins except nicotinic acid. Riboflavin deficiency is predominant.

The use of a cheap source of animal protein in the shape of sea fish, poultry and green vegetables as a source of vitamin is recommended. Consumption of groundnut is also recommended to meet the protein requirement.

5. Inquiry into metabolism of vitamin A under Dr. K. Rajagopal at the All-India Institute of Hygiene & Public Health, Calcutta.

I. VITAMIN A IN URINE.

Studies of vitamin A excretion with urine were conducted on twenty-eight lobar pneumonia cases. In sixteen cases vitamin A was detected in the urine ; of this only in eleven cases protein was present in urine. In the remaining five cases no protein was detected by the common tests. Purine was always in higher concentrations and was found to decrease with lowering of w.b.c. count. No parallelism was, however, observed between vitamin A excretion and purine excretion : For example, one case had 14,500 w.b.c. count and vitamin A was present 75 I. U. per 100 c.c., when the w.b.c.count fell to 11,000 there was no vitamin A detectable in the urine. In another case having a w.b.c. count of 13,000 no vitamin A was detected. In both the cases the uric acid concentrations were high, in the former case it was 41·5 mg./per cent and in the latter 48·7 mg./per cent (the normal range of uric acid being approximately 35 mg./per cent). It was observed that even in absence of protein, vitamin A was excreted and protein-free urine with high purine can take up vitamin A, but this property was lost when it was boiled for some time. When urine was freed from purines and other salts by chromatography with Lloyd's reagent, it lost its property of dissolving vitamin A. Investigations on the influence of serum-proteins on dissolving vitamin A are in progress. For this purpose different plasma fractions are being prepared in the undenatured state by 'ethanol fractionation'.

II. BLOOD, VITAMIN A AND BLOOD, CAROTENE.

(*a*) In course of estimation of vitamin A and carotene in ox-plasma and its different fractions, Yudkin's (*Biochem. Jour.*, **35**, p. 551, 1941) method was attempted; but it was found that single petrol-ether extraction following precipitation with alcohol was not sufficient and at least three extractions were necessary for complete extraction. The method for estimation was found to give moderately accurate values in human cases, where vitamin A content is higher or nearly equal to carotene content (viz. 1·15 I. U. vitamin A and 0·95 μg. of carotene per c.c. of plasma). In grazing cattle, in which the plasma-carotene values are high (viz. 12·3 μg./c.c.) with very low vitamin A content (viz. 0·4 I. U./c.c.) this method gave misleading values. Yudkin's method was also found unreliable as the time required for the production and fading out of blue colour with $SbCl_3$ reagent was different for vitamin A and carotenes. In some cases, the galvanometer reading for $SbCl_3$ reaction with Evelyn photo-electric colorimeter gradually began to rise from the moment of addition of the $SbCl_3$ reagent for about fifteen minutes. Oxidized products of carotene, which are responsible for this abnormality, also produce higher blue values than pure carotenes (*Jour. Biol. Chem.*, **168**, p. 83, 1947). Chromatographic separation was also not found practicable due to the long time required for coping with numerous samples and very low amounts of vitamin A present in the plasma fractions. To overcome this difficulty, the absorption spectra of the petrol-ether extract of plasma or plasma fraction were recorded in the range 320μ

to 500 μ and compared with standard absorption spectra of mixtures of carotene and vitamin A of high degree of purity, in different proportion. As no data for absorption spectra of vitamin A in petrol-ether were found in literature available here, absorption spectra of vitamin A, in petrol-ether and in cyclohexane were taken simultaneously both in a Spekker spectro-photometer and in a Beckmann's photo-eletric spectro-photometer. Excepting the general absorption in petrol-ether in the lower region up to 274 μ, the two spectra were found to be almost similar for all practical purposes.

Carotene of a high degree of purity was prepared from fresh carrot, by extraction with freshly distilled petrol-ether, saponification with alcoholic caustic potash and finally purified by chromatography. To prevent oxidation during chromatography, the absorbent magnesium oxide was alternately calcined in a current of nitrogen and put under vacuum three times, and all subsequent operations were performed in an atmosphere of nitrogen.

Vitamin A was purified from non-saponifiable fraction of halibut liver oil containing 52,640 I. U. per gramme, by dissolving in methyl alcohol, cooling with carbon-dioxide snow to crystallize out the sterols and filtering off at the same low temperature. In calculating vitamin A content in a mixture with carotene it was found necessary to subtract from the observed absorption maxima at 328 μ the absorption maxima at 450 μ divided by 6·31. For calculating carotene content, no subtraction was necessary, as vitamin A has almost no absorption at that region. This method is very promising and has been in use in connection with the present work.

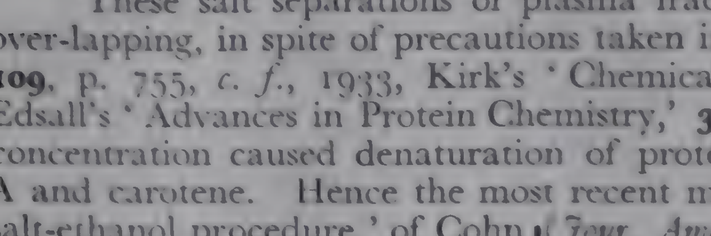
(*b*) Though it is certain that carotene and viatmin A are in some sort of molecular association with some protein fractions of plasma (*Biochem. Jour.*, **39**, p. 63, 1945), reports on the exact nature of the protein concerned are conflicting. Pett and Le Page (*Jour. Biol. Chem.*, **132**, p. 585, 1940) suggested globulin to be the protein concerned, whereas Dzialeszynski *et al.* (*Biochem. Jour.*, **39**, p. 63, 1945), strongly support the view that albumin is the protein concerned. Attempts were made to fractionate the plasma-protein by salting out with ammonium sulphate. Concentrated solution of ammonium sulphate was allowed to diffuse into the plasma very slowly from a sintered crucible (No. 2) in order to prevent any localized high concentration of ammonium sulphate, and fractions separated at different salt concentration were separated. In three samples, 100 c.c. of plasma (citrated) were fractionated and vitamin A and carotene were found to be distributed in the following way :—

Samples.		Total per 100 c.c.	Fibrinogen.	Globulin.	Albumin.
I	Vitamin A	54 I.U.	*Nil*	36 I·U.	16 I.U.
	Carotene	970 μg.	*Nil*	560 μg.	410 μg.
II	Vitamin A	48 I.U.	*Nil*	32 I.U.	16 I.U.
	Carotene	720 μg.	*Nil*	420 μg.	300 μg.
III	Vitamin A	84 I.U.	*Nil*	54 I.U.	40 I.U.
	Carotene	650 μg.	*Nil*	380 μg.	270 μg.

Considering the higher amount of albumin in the plasma, the carotene and vitamin A seem to be more concentrated in the globulin fraction. Similar results were obtained with sodium sulphate separation.

These salt separations of plasma fractions were found to cause much over-lapping, in spite of precautions taken in adding salts (*Jour. Biol. Chem.* **109**, p. 755, *c. f.*, 1933, Kirk's 'Chemical Determination of Proteins' in Edsall's 'Advances in Protein Chemistry,' **3**, p. 160). Moreover, high salt concentration caused denaturation of proteins, with liberation of vitamin A and carotene. Hence the most recent method of 'low temperature-low salt-ethanol procedure' of Cohn (*Jour. Amer. Chem. Soc.*, **68**, p. 459, 1946). was attempted. Here all operations had to be carried out at temperature between 0°C. and 5°C. Citrated plasma kept at 0° C. was treated with aquous ethanol (53·3 per cent) and sodium-acetate acetic acid buffer at temperatures −3°C. to −5°C. according to the Diagram, and the precipitates were separated by centrifugation. All operations were being carried out in a cold room at 3—5°C. and the centrifuge tube with contents were heat-insulted with cotton placed in cups and the whole were pre-cooled to about −8°C. with freezing mixture. This was found necessary to counteract the rise of temperature during centrifugation (about 3°C.). The pH was determined with glass-electrode in aliquots. At present only the main fractions are being investigated. The albumin fraction (precipitate V) thus obtained was found to contain almost no carotene. Further work on sub-fractionation is in progress.[6]

DIAGRAM



There is an unpublished report (K. C. D. Hickman) that vitamin A in human plasma was found concentrated in fraction III-O, which is a sub-fraction of fractions (II and III) (Mulford, *Ann. Rev. Physio.*, **9**, p. 335, 1947). This fraction is also said to be identical with *X*-protein of Pederson containing 75 per cent lipid, obtained by electro-phoresis (Pederson, 1945, 'Ultra-centrifugal studies on Serum and Serum Fractions', Upsala). Moreover, this particular type of lipo-protein was reported to be absent in all animal plasma hitherto investigated. The ethanol separation method is expected to provide some clue to this problem.

(*c*) Carotene content of serum was found to diminish in course of time when the serum was kept at room temperature even in an atmosphere of nitrogen. This diminution was accompanied by precipitation of proteins of serum. This destruction of carotene as well as precipitation of proteins could, however, be greatly reduced when cyanide is added to the serum. This phenomenon is being investigated in order to study the breakdown products of carotene formed in serum.

(*d*) Recent investigations of Wald (*Jour. Gen. Physiol.*, **32**, p. 367, 1949) shows that vitamin A-aldehyde remains in chemical combination with protein in water soluble form in the visual system. Absorption spectra of ox-plasma extract in petrol ether, however, showed no inflexion at $369{\cdot}5\ \mu$ at which the absorption maxima of vitamin A-aldehyde occurs. Studies on human cases are in progress.

Vitamin A-aldehyde is being prepared from freshly prepared vitamin A alcohol (described above) by oxidation with MnO_2 prepared from $KMnO_4$ and $MnSO_4$ (Ball *et al.*, *Biochem. Jour.*, **48,** p. 506, 1948). By Wald's method (*Jour. Gen. Physiol.*, **31,** p. 500 1948), which was first attempted, oxidation could not be controlled and products other than vitamin A-aldehyde were obtained.

Vitamin A-aldehyde will be used for studying its behaviour towards different protein fractions of plasma.

6. Inquiry on the preparation of anti-rachitic substances under Professor B. V. Bhide at the Maharaja Pratap Sinh Chemical Laboratory, S. P. College, Poona.

Preparation of alkyl-pimelic acids was undertaken. Several methods were explored but had to be given up due to unsatisfactory yields. Cholesterol and calciferol phosphoric esters were prepared and sent to Dr. Patwardhan, Director, Nutrition Research Laboratories, Coonoor. Since April the starting material quinnitol has been prepared in quantity and the complete technique of high pressure hydrogenation set up. Calciferol was hydrogenated to octa-hydro derivative but was found to have no anti-rachitic properties. Evidently the triene system is essential for anti-rachitic properties. It is expected that the work as initially planned will be completed by the end of March 1950.

7. Inquiry into the effects of malnutrition on the physique and more particularly on the blood picture including hæmoglobin and plasma-protein concentration as also the treatment thereof with high-protein diet, protein hydrolysates, etc., under Professor (Miss) S. Chaudhuri and Dr. K. Mitra at the Lady Hardinge Medical College Hospital, New Delhi.

It had been noticed during the War period and during the following years, especially since the influx of refugees from West Punjab into Delhi, that the number of cases of nutritional deficiency diseases including anæmias admitted to the Medical Unit of the Lady Hardinge Medical College Hospital had increased considerably. Large numbers of them had œdema and anæmias of severe degrees in whom plasma-protein concentration was found to be low and such cases responded considerably to high-protein diet and on oral administration of concentrated protein hydrolysates.

The object of this inquiry was to determine the effects of lowered state of nutrition on the quality of blood, i.e. to find out if there was any correlation between inadequacy of protein in the diet, the plasma proteins and the degree of anæmia both as regards the hæmoglobin and red blood cells.

The investigation could not be started before the lst of June, 1949 as the appointment of the Research Assistant had to be made after the usual procedure. During the period of four and a half months up to 15th October, 1949 thirty-five adult female anæmia cases and ten infants and children with malnutrition have been investigated, at the same time thirty normal adult females had to be investigated as controls.

These patients mostly came from lower middle and poor classes, and included vegetarians and non-vegetarians; but the so-called non-vegetarians consumed very little meat and neither of them took any milk in their routine dietary. In selecting these cases those who showed hæmoglobin percentage of fifty or above by Tallquist's paper-method were not included in this series, detailed dietary history was taken and thorough clinical examination was done in each case. Hæmatological examination consisted of estimation of hæmoglobin by Sahli's hæmoglobinometer which was standardized, total red blood cell count on Naubauer's counting chamber, mean corpuscular volume in Wintrobe's tubes using electric centrifuge with 2,500 r.p.m., also M.C.H., reticulocyte count and blood picture, etc. Plasma protein determinations were carried out in all cases in children and infants by van Slyke's copper sulphate specific gravity method and in adults by above method as well as by micro-Kjeldahl nesselerization method and thereby albumin : globulin ratio was determined. These examinations were done in each case, on admission and before discharge. Blood volume was done by Evan's blue dye method in ten cases to correlate the total plasma protein values with the blood volume.

In view of the prevailing opinion that lowered gastric acidity interferes with the assimilation of nutrients present in the food, gastric analysis was done in each case including response to histamine whenever necessary, the subjects chosen did not include those suffering from any clinical conditions which may produce anæmia; out of 35 cases 15 were pregnant.

Patients were put on high-protein diet, meat extracts, protein hydrolysates orally, in order to find out the response in plasma protein concentration, hæmatological values and improvement in their clinical condition, particularly nutritional œdema.

It is not possible to draw definite conclusions on the results so far obtained but the following interesting points have been noticed :—

1. The caloric value of the diet consumed by these patients prior to admission was definitely very low, ranging from 1,000 to 1,600 calories, the protein content of the diet was hardly 60 g. per day in 80 per cent of adult patients even on an optimistic estimate. The body-weight varied from 112 lb. to 120 lb. in adult anæmia cases.
2. In two-thirds of the subjects the plasma protein concentration did not come up to even 5 g./100 c.c., minimum observed was 2·1 g./100 c.c. on admission.
3. Albumin : globulin ratio ranged from 0·7 : 1 to 3·5 : 1, average being 2 : 1 before treatment.
4. An apparent correlation could be discovered between the plasma proteins and hæmatological values. Albumin : globulin ratio was lowered in such cases in spite of the fact that there was no urinary excretion of albumin.
5. Even in fifty per cent of the so-called normal adults, plasma-proteins concentration did not come up to 7 g./100 c.c., figure as low as 4·5 g./100 c.c. has been recorded, albumin : globulin ratio was in the order of 2 : 1.

6. Inclusion of animal proteins in the diet or administration of protein hydrolysates orally was followed by definite improvement in the protein content in the plasma and hæmatological values and favourable response in albumin : globulin ratio. Definite improvement could be noticed clinically, especially in subjects with œdema.
7. Medication with iron or liver extract or folic acid alone or in combination was not so effective as when proteins were added.
8. Blood volume figures were definitely low in anæmia cases, it is significant to note that low figures of protein concentration in blood was associated even with low blood volume in these subjects.

8. Inquiry on the biosynthesis of nicotinic acid under Dr. S. Banerjee at the Presidency College, Calcutta.

Part I.—BIOSYNTHESIS OF NICOTINIC ACID BY GERMINATING PULSES.

Estimation of nicotinic acid plus *nicotinamide, nicotinuric acid (Nicotinylglycine) and trigoneline in dry and germinated seeds.*—Nicotinic acid and nicotinamide were estimated in aliquots of the filtrate obtained after filtering a normal hydrochloric-acid extract of the material, following a method similar to that used by Banerjee, Ghosh and Bhattacharya (1948). Nicotinuric acid in dry and germinated seeds was determined by a method similar to that used by Perlzweig, Levy and Sarett (1940) for urine. Trigonelline in seeds was determined by a modification of the method followed by Sarett (1943) for urine. The results are given in Tables I, II and III.

Estimation of alkali-labile precursor of nicotinic acid.—Five g. of the material were crushed and extracted with 0·1N HCl for one hour on a boiling water bath. After cooling, the pH was adjusted to 4·5 to 5·0 when the protein precipitated out. The volume was made up to 100 c.c. and filtered nicotinic acid was estimated in an aliquot of the filtrate. Other aliquots were treated with alkali so as to make the concentration of sodium hydroxide in the solution equal to 0·3 per cent, and kept for five minutes at room temperature (30°C.). This was acidified at the end of five minutes' period, and nicotinic acid estimated. The difference of the two values gave the value for the so-called precursor calculated as nicotinic acid. The results are given in Table IV:—

TABLE I.

Effect of germination on the nicotinic acid plus *nicotinamide content of cereals and pulses.*

(Figures are expressed in mg. per 100 g. of the material).

Name of seed.	Days of germination.					
	0	1	2	3	4	5
Gram (*Cicer arietinum*) ..	1·43	1·54	1·68	1·67	2·43	2·50
Pea (*Pisum sativum*)	1·29	1·57	1·62	2·44	2·50	2·44
Mung (*Phaseolus mungo*) ..	2·23	4·66	4·95	5·38	6·22	7·88
Kalai (*Phaseolus radiatus*) ..	1·45	1·49	2·22	3·68	..	3·27
Barley (*Hordeum vulgare*) ..	2·89	..	..	2·56	3·14	..
Wheat (*Triticum vulgare*) ..	1·97	1·84	1·90	2·23	2·23	..

TABLE II.

Effect of germination on the nicotinic acid, nicotinuric acid and trigonelline content of Phaseolus mungo.

(Figures are expressed in mg. per 100 g. of the material).

Days of germination.	Nicotinic acid *plus* nicotinamide N HCl extract.	Nicotinuric acid calculated as nicotinic acid.	Trigonelline as hydrochloride.
0	2·23	1·33	307·4
1	4·66	1·10	301·7
2	4·95	1·23	291·4
3	5·38	3·06	246·3
4	6·22	3·36	243·5
4	7·88	..	235·0

TABLE III.

Effect of different substances on the nicotinic acid plus *nicotinamide content of germinating* Phaseolus mungo.

(Figures are expressed in mg. per 100 g. of the material).

	Substances used.	Days of germination.			
		1	2	3	4
(*a*)	Water (control) ..	2·92	2·78	4·24	..
	Ornithine ..	2·48	2·68	3·58	..
	Valine ..	2·54	..	4·26	..
	Methionine ..	2·66	..	4·40	..
(*b*)	Control ..	2·70	3·10	3·78	3·68
	Phenylalanine ..	2·20	2·06	2·76	2·70
	Cystine	2·40	1·98	2·50	2·52
	Isoleucine ..	2·18	2·00	2·50	2·80
(*c*)	Control ..	4·34	4·70	5·00	5·06
	Chloretone ..	4·14	6·50	5·92	6·74
	Menthol	2·90	3·04	3·62	..
	Camphor ..	2·96	3·40	3·50	..
	Barbitone ..	2·52	3·34	3·34	3·40
(*d*)	Control ..	3·38	4·46	4·78	4·64
	Tryptophane ..	2·96	4·68	4·70	5·58
	Phyridoxine ..	2·30	4·16	4·40	4·56
	Tryptophane *plus* Pyridoxine	1·86	3·58	3·92	4·66
	Choline	2·12	3·56	3·64	3·94

Experiments in groups (*c*) and (*d*) were performed with a different strain of *Phaseolus mungo* than that used in groups (*a*) and (*b*). All samples in each individual group have been run concurrently and afford good comparison.

TABLE IV.

Effect of germination on the alkali-labile precursor of nicotinic acid in cereals and pulses.

(Figures are expressed in mg. per 100 g. of material).

Seeds used.	Days of germination.				
	0	1	2	3	4
Barley (*Hordeum vulgare*) ..	2·50	..	2·50	2·23	2·12
Wheat (*Triticum vulgare*)	2·23	1·80	..	1·70	1·51
Pea (*Pisum sativum*) ..	0·17	0·15	0·00	0·01	0·10
*Paddy (*Oryza sativa*) ..	1·66	..	..	..	..
Brewers' yeast tablets ..	1·96	..	..	..	..

*Paddy could not be satisfactorily germinated under the stated conditions.

Results obtained indicate that the nicotinic acid value was considerably increased during the course of germination in pea, gram, mung and kalai. With barley and wheat, however, the increase in the nicotinic acid value after three to four days of germination was not very high.

The trigonelline content of *Phaseolus mungo* shows a marked decrease as the germination proceeds. *Phaseolus mungo* is very rich in trigonelline, and a decrease in the trigonelline content during the course of germination may be the cause of the rise in the nicotinic and nicotinuric acid values during the process.

The decrease in the trigonelline content, however, is many times the increase in the nicotinuric-acid and nicotinic-acid contents during the course of germination. Further work is necessary to account for the decrease in the trigonelline content of pulses during germination.

From Table III it appears that all the compounds examined, except chloretone and tryptophane, either do not affect the synthesis or exert a positive inhibitory influence on the production of nicotinic acid during germination.

The positive beneficial action of chloretone and tryptophane rather than the amino acids, such as ornithine, makes it difficult to reconcile our finding with Guggenheim's postulation regarding the synthesis of nicotinic acid in plants *via* the ornithine pathway. The nicotinic-acid content of the seeds of *Phaseolus mungo* germinated with chloretone and tryptophane is increased during the process of germination. This increase does not seem to be due to conversion of chloretone or tryptophane into nicotinic acid as these substances are dissimilar and the amount of increase in the nicotinic-acid content is only a very minute fraction of these substances used during germination. It may be suggested that chloretone and tryptophane are acting as stimulators rather than precursors of nicotinic acid.

From Table IV it appears that in wheat and barley, the alkali-labile fraction undergoes a definite decrease as the germination proceeds. In pea, the amount of the latter fraction present is very small, and though present in small amounts in dry powder, seems to have disappeared in germinated condition. Pea seems to be a representative of some other pulses, which also contain only a very small amount, if any, of this alkali-labile fraction.

It is of interest to note in this connection that we could not find an increased amount of nicotinic acid in HCl extracts of maize when treated with 0·3 per cent sodium hydroxide in the specified manner. In this respect maize, a cereal, seems to be at variance with other cereals examined, i.e. wheat, barley and paddy. This finding is in accord with the well-known pellagragenic action of maize.

It is interesting to note that a sample of Squibb's brewers' yeast tablets furnished evidences of the presence of a comparatively small amount of alkali-labile fraction.

Dependence of nicotinic acid value on variation in strain and other condition.—Numerous experiments have shown that the nicotinic acid content of cereals and pulses are to some extent affected by the origin, storage and variations in strain of the seeds in question. Similarly, the increased amount of nicotinic acid elaborated by the seeds on germination is also dependent to some extent on such conditions, viz. temperature, humidity and exposure to light. Notwithstanding deliberate attempts to standardize the conditions of germination, certain variations, mainly affecting temperature and atmospheric humidity, were inevitable from week to week. In Table III, therefore, the samples assayed have been classified separately according to the number of samples run concurrently. Samples thus run afford good comparison amongst themselves, but it is safer not to compare results tabulated in group (*a*) with those in group (*d*).

Part II.—BIOSYNTHESIS OF NICOTINIC ACID BY GUINEA PIGS.

Urinary excretion of nicotinic acid and N^1-methyl-nicotinamide by guinea pigs.–Healthy adult guinea pigs, fed on a normal colony diet of germinated gram and grass, were kept in individual metabolism cages and urine collections were made on two successive days. The nicotinic acid content of urine as determined after hydrolysis by N HCl were determined. The N^1-methyl-nicotinamide was also determined according to the method of Huff and Perlzweig (1947). From the third day, for two days test doses of 20 mg. of nicotinic acid were given to each animal, and the nicotinic acid and N^1-methyl-nicotinamide contents of the urine collected every day for subsequent two days were evaluated. The results are given in Table V:—

TABLE V.

Effect of a dose of nicotinic acid on the urinary excretion of nicotinic acid and N^1-methyl-nicotinamide in guinea pigs.

Twenty mg. of nicotinic acid were fed after the collection of 2nd day's urine. (The results are expressed in micrograms.)

	1st day.	2nd day.	3rd day.	4th day.
Nicotinic acid in urine ..	82·5	67·0	20·70	19·80
N^1-methyl-nicotinamide in urine	24·0	35·0	32·0	43·0

While the nicotinic acid content of urine showed a steep rise, a fluorescent compound estimable as N^1-methyl-nicotinamide did not show any remarkable increase. It was, therefore, concluded that N^1-methyl-nicotinamide did not constitute the main excretion product of nicotinic acid metabolism in guinea pigs. The estimation of N^1-methyl-nicotinamide-like compound was, therefore, dispensed with in subsequent experiments.

The growth of guinea pigs kept on a 64 *per cent corn diet.*—A group of six guinea pigs were fed a 64 per cent barley diet consisting of whole powdered barley 64, gram 20, casein 12 and salts 4. Another group of 34 received the corn diet consisting of maize meal 64, gram 20, casein 12 and salts 4. All the animals received a supplement of 5 mg. of vitamin C daily and four drops of a concentrate of vitamins A and D. The animals were weighed on alternate days.

The animals on corn diet were divided into three groups, each group consisting of six animals. Group I received 10 mg. of nicotinic acid and those of group II 20 mg. of dl-tryptophane daily. The animals of group III did not receive either nicotinic acid or tryptophane. The animals in each group excepting those receiving a supplement of nicotinic acid were put in metabolism cages twice a week, and their urine was collected under toluene of 24 hours. The intake of food during the 24-hour period was noted. The amount of nicotinic acid excreted in the urine was determined after hydrolysis with normal hydrochloric acid (Banerjee, Ghosh and Bhattacharya, 1948).

TABLE VI.

Growth of guinea pigs kept on a 64 *per cent corn diet.*

Description of diet*.	Average gain in weight in grams during:				
	1st week.	2nd week.	3rd week.	4th week.	5th week.
1. Barley diet (6 animals)	31·3	23·5	12·9	18·0	17·0
2. Corn diet (12 animals)	16·0	1·0	−8·0	−13·0	−16·0
Corn diet (2) + 10 mg. of nicotinic acid (6 animals	16·0	7·0	−13·0	9·0	4·0
Corn diet (2)+20 mg. of dl-tryptophane (6 animals)	6·0	8·0	−18·0	1·0	−17·0

*Supplements to the basal diet, e.g. nicotinic acid and tryptophane, were given from the end of the 3rd week and continued afterwards.

TABLE VII.

Percentage excretion of ingested nicotinic acid in guinea pigs fed barley diet, corn diet, and corn diet plus 20 *mg. of dl-tryptophane.*

Description of diet.	Average percentage of nicotinic acid ingested with food as excreted in urine.				
	1st week.	2nd week.	3rd week.	4th week.	5th week.
1. Barley diet ..	..	8·0	..	..	5·7
2. Corn diet ..	11·2	11·2	11·9	10·6	8·1
Corn diet (2) *plus* dl-tryptophane daily	..	10·2	9·0	11·6	9·8

TABLE VIII.

Relative incidence of mortality in guinea pigs fed barley diet and corn diet.

Description of diet.	Percentage of animals showing irregular growth curves and consequently rejected.	Percentage of animals showing good growth curves at the start but succumbing in subsequent weeks.				
		1st week.	2nd week.	3rd week.	4th week.	5th week.
1. Barley diet . .	25	. .	. .	. .	. .	. .
2. Corn diet . .	44	. .	2·3	4·6	9·2	16·0

Estimation of total body nicotinic acid of normal guinea pigs and of guinea pigs kept on a synthetic nicotinic acid free diet for 3 weeks.—Eight guinea pigs fed barley diet *ad libitum* for two days were made familiar by stages with a synthetic nicotinic acid free diet, consisting of sucrose 45, vitamin-free casein 30, gum accacia 15, Steenbock salt mixture 4 supplemented with vitamins D, E, C and those of the B complex excepting nicotinic acid, in levels suggested by Booth, Elvehjem and Hart (1949). Five days after they had been allowed to live solely on the synthetic diet, four animals were killed, and the total nicotinic acid content of the carcass determined.

The remaining four animals were housed in metabolism cages. They received weighed amounts of the diet *ad libitum* and were fed 20 mg. of dl-tryptophane per animal per day. Urine and fæces were collected daily, and the amount of nicotinic acid thus excreted were estimated.

After a period of three weeks on this régimen, all the animals were killed and the total nicotinic acid in the body of each individual animal determined. The results are given in Table IX :—

TABLE IX.

Total body nicotinic acid content of guinea pigs, before and after a 3-week régimen on a synthetic nicotinic acid free diet with supplement of tryptophane.

Description of group.	Total body nicotinic acid at start of experiment, mg.	Total body nicotinic acid at the end of three weeks, mg.	Nicotinic acid ingested during this period, mg.	Nicotinic excreted, mg.	
				In urine.	In fæces.
Four animals kept on synthetic diet and 20 mg. of tryptophane daily	*44·20	40·0	0·98	3·00	1·39

*Calculated on the basis of 36·3 microgram of nicotinic acid per g. of body-weight of the four animals killed at the start of the experiment.

The results presented in Table VI clearly indicate that a diet rich in yellow corn does not constitute a complete diet for the growth of guinea pigs. Animals fed barley diet containing 64 per cent powdered whole barley rapidly grow in weight and maintain growth up to and after the 5th week. The animals kept on corn diet show a much lesser gain even from the first week, and during the 3rd week begin to lose weight. The animals kept on corn diet without supplement of nicotinic acid or tryptophane continue to fall in weight during subsequent weeks. Those receiving tryptophane show the same tendency. It is hardly possible from the data presented in Tables VI and VII to draw any distinction between the nicotinic acid status of corn diet fed animals, with and without supplement of tryptophane, and the growth characteristics or percentage excretion. The group of animals receiving 10 mg. daily supplement of nicotinic acid from the end of the 3rd week, however, show definite signs of improvement so far as growth is concerned. The growth rate thus achieved is never comparable with the growth rate in animals receiving barley diet. It is, therefore, clear that the deleterious effects of corn on the growth of guinea pigs cannot be solely due to a deficiency in nicotinic acid. The growth-depressing factor in maize as suggested by Wooley (1946) and Kodicek, Carpenter and Harris (1946) may be partly responsible for the inhibition of growth. This view is further strengthened by the fact that none of the animals kept on the nicotinic acid free diet for three weeks died during this period.

The results also indicate that guinea pigs do not excrete increased amounts of nicotinic acid or derivatives when fed tryptophane. It appears, therefore, that guinea pigs are either incapable of synthesizing nicotinic acid, or that the biosynthesis, if any, does not involve tryptophane either as a starting point or as an intermediary pathway. The significance of the absence of tryptophane in maize is, therefore, lessened to a considerable extent when maize is applied as a pellagragenic feed to a guinea pig, in which the presence or absence of an excess of tryptophane would be unimportant so far as the elaboration of nicotinic acid is concerned.

The complete absence of any mechanism for the biosynthesis of nicotinic acid in guinea pigs, with or without the involvement of tryptophane is amply demonstrated in the experiments with synthetic diet fed animals. A 30 per cent level of casein in diet would itself have an anti-pellagra effect in rats (Krehl, Popley, Sarma and Elvehjem, 1945). A balance sheet of nicotinic acid excretion and conservation clearly shows that no nicotinic acid has been synthesized in the body of guinea pigs kept on the synthetic diet supplemented by 20 mg. dl-tryptophane daily. The considerable amount of nicotinic acid excreted in the urine and fæces seem to be endogenous in origin.

Failure of the guinea pig to excrete the main bulk of excretion as N^1-methyl-nicotinamide is noteworthy. This is in confirmation of the findings of Handler (1944) who observed no toxic effects in guinea pigs after a heavy dose of nicotinic acid. The latter worker believes that toxicity under similar circumstances in rats is caused by a depletion of labile methyl groups caused by the heavy demand for the same in order to methylate the unusual quantities of nicotinic acid ingested.

Harris and Raymond (1939) reported to have found that the urinary excretion of nicotinic acid in their animal ceased altogether after 3 to 4 weeks on a modified Goldberger diet. We could not, however, confirm the above, in so far that our animals continued to excrete considerable though lesser amounts of nicotinic acid even in the 5th week. The diet used by Harris and Raymond contains 13 to 14 micrograms of nicotinic acid per g. Assuming an average intake of 15 g. of food, the daily ingested nicotinic acid would amount to about 200 micrograms. Our animals on synthetic nicotinic acid free diet which ingested only 16 to 17 micrograms of the vitamin daily still excreted considerable amounts of nicotinic acid in the urine and fæces after a 3 week régimen.

9. Inquiry on the physiological effects of dietary protein under Dr. M. Damodaran at the National Chemical Laboratory, Poona.

Isoleucine in hæmopoiesis.—Only one aspect of the inquiry on the physiological effects of dietary protein, viz. the role of amino acids in hæmopoiesis has so far been taken up. In continuation of the work done on tryptophane the role of isoleucine in hæmoglobin and r.b.c. regeneration was studied. The experimental procedure adopted was that previously described in detail by Yeshoda and Damodaran (1947).

White rats were made anæmic by injection of phenyl-hydrazine and recovery from anæmia studied on two diets, one lacking in isoleucine and the other containing this amino acid. The isoleucine deficient diet contained as the sole source of nitrogen horse globin which is known to be completely lacking in isoleucine (Brand and Grantham, 1946). The globin was prepared from horse hæmoglobin by a modified technique combining the main features of the methods described by Schulz (1898) and Anson and Mirsky (1930). The isoleucine required for supplementing this diet was prepared *via* the copper salt (Damodaran, 1931), either from casein itself or from casein phosphopeptone (Damodaran and Ramachandran, 1941). The isoleucine free basal diet had the following composition :—

	per cent
Horse globin	18
Starch (Tapioca)	40
Sugar	20
Butter fat (melted)	17
Salt mixture (Steenbocks)	5

The results are summarized in the Table which gives the average increases in r.b.c. and hæmoglobin, and the average weight changes in a typical experiment carried out on six groups, each containing eight animals, and given varying quantities of isoleucine supplements.

It will be seen from the Table that white rats receiving more than 20 mg. of isoleucine regained normal r.b.c. and hæmoglobin values in eight days (from the day of acute anæmia, i.e. twelve days after the injection of phenyl-hydrazine), while the animals receiving either no isoleucine or only 10 mg. remained anæmic. Animals of this group, it was found, regained normal values only on the 17th day. It will also be seen that, while 20 mg. isoleucine caused normal blood regeneration, this quantity was not sufficient to maintain body weight, animals receiving up to 40 mg. of the amino acid still showing pronounced loss in weight. With a supplement of 100 mg. of isoleucine normal increases in weights were obtained.

TABLE.

Diet.	Isoleucine dosage mg./rat/day.	Weight in g.		R.b.c. (millions/c.mm.).				Hæmoglobin (g./100 c.c.).		
		Days.		Difference, per cent + or −	Days.		Increase, per cent +	Days.		Increase per cent +
		0	10		0	8		0	8	
Globin diet	0	121·10	106·90	−11·50	3·66	5·32	+45·17	9·50	12·30	+29·06
Globin diet + isoleucine	10	117·60	106·30	− 9·90	3·61	5·58	+55·15	9·50	12·60	+32·43
	20	124·60	114·60	− 8·20	3·47	6·37	+83·95	9·40	14·60	+56·06
	20 mg. (by injection)	112·50	100·50	−11·30	3·75	6·45	+72·39	9·80	11·10	+44·82
	40	125·60	118·50	− 5·90	3·42	6·37	+88·09	9·30	14·60	+57·13
	100	116·30	132·10	+13·90	3·67	6·55	+72·39	9·40	14·40	+52·73

It may be concluded from the results obtained that (*i*) isoleucine is essential for normal hæmopoiesis and (*ii*) the requirements in isoleucine for hæmopoiesis have to be met before the amino acid is utilized for growth.

Maintenance of animals at different levels of protein intake.—The various proteins required for this work are under preparation.

List of papers published on nutrition research.

BANERJEE, S., and BANERJEE, R. Studies on the biosynthesis of nicotinic acid. Part I. Biosynthesis of nicotinic acid by germinating pulses. (*I. J. M. R.*, **38**, April 1950).

BANERJEE, S., BANERJEE, R., and CHANDI CHARAN DEB. Studies on the biosynthesis of nicotinic acid. Part II. Biosynthesis of nicotinic acid by guinea pigs (*I. J. M. R.*, **38**, April 1950).

DIKSHIT, P. K., and RANGANATHAN, S. The vitamin D content of butter and ghee (clarified butter) (*I.J.M.R.*, **38**, January 1950).

PATWARDHAN, V. N., MUKUNDAN, R., RAMASASTRI, B. V., and TULPULE, P. G. Studies in protein metabolism. The influence of the dietary protein in the urinary nitrogen excretion (*I.J.M.R.*, **37**, July 1949).

RAMALINGASWAMI, V., and MENON, P. S. Folic acid in nutritional macrocytic anæmia (*I.J.M.R.*, **37**, October 1949).

RANGANATHAN, S., and PATWARDHAN, V. N. Alkaline phosphatase in erythrocytes (*I.J.M.R.*, **37**, April 1949).

SUNDARARAJAN, A. R. Comparative nutritive value of milk and curd (*I.J.M.R.*, **35**, January 1950).

TULPULE, P. G., and PATWARDHAN, V. N. Influence of pyridoxine on fat metabolism. The composition of tissue lipoids in fat and pyridoxine deficiency (*I.J.M.R.*, **38**, January 1950).

REPORT OF THE 18TH MEETING OF THE NUTRITION ADVISORY COMMITTEE.

Held in Calcutta on the 7th and 8th July, 1949.

COMPOSITION OF THE COMMITTEE.

Members present.

Dr. G. Sankaran. (*Chairman*).
Professor S. P. Agharkar.
Dr. S. Banerji.
Professor M. Damodaran.
Dr. B. C. Guha.
Dr. (Miss) R. Karnad.
Dr. K. Mitra.
Dr. V. N. Patwardhan. (*Secretary*).

Dr. K. P. Basu, Dr. V. Subrahmanyan, Mr. B. N. Adarkar, Dr. R. B. Lal and Dr. C. O. Karunakaran were unable to be present.

Visitors.

Lieut.-Colonel C. K. Lakshmanan.
Dr. M. V. Radhakrishna Rao.
Dr. K. Rajagopal.
Dr. C. R. Das Gupta.
Miss G. V. Subama.
Dr. P. C. Sen.
Mr. B. N. Banerji.
Dr. S. L. Hora.

Dr. C. G. Pandit, Secretary, Indian Research Fund Association, also attended the meeting.

1. To consider the report of the Nutrition Committee of the Food and Agricultural Organization of the United Nations held in Baguio, Philippines, from 23rd to the 28th February, 1948 on the nutrition problems of rice-eating countries in Asia and to note the statement made by Dr. V. N. Patwardhan, M.Sc., Ph.D., A.I.I.Sc., Director, Nutrition Research Laboratories, Coonoor, and Chairman of the Committee, while presenting the report at the F.A.O. Rice Meeting held in Baguio, Philippines, from the 1st to 13th March, 1948.

The Committee discussed the report of the Nutrition Committee held at Baguio, Philippines, from the 23rd to 28th February, 1948 and found themselves in general agreement with the recommendations made there. The Committee, however, felt it necessary to lay emphasis on some aspects with special reference to India in order that the authorities concerned may have at their disposal expert advice on some of the problems in case immediate action is contemplated. The resolutions adopted by the Committee on several of these subjects are as follows :—

1. The Committee agree with the recommendations made by the Baguio Committee about the desirability of ensuring a minimum level of thiamine in rice marketed for consumption. The Committee are fully aware of the problems and difficulties involved and are of the opinion that the improvement of milling practices would result in the production of rice with the desired thiamine content. In order that a check on the quality of rice may be effectively maintained, it is desirable to develop a rapid and reliable method of thiamine estimation for use in mills and laboratories. The Committee recommend that nutrition workers in India should take up this problem for investigation according to a co-ordinated plan which may be considered at its next meeting.

2. The production, marketing and consumption of rice containing 1·5 μg. of thiamine per gramme presents problems of storage, palatability, lowered digestibility and the adverse effect of increased phytin content on the nutritive value of rice. The Committee, therefore, recommend that investigations on these should be undertaken at an early date.

3. There is immediate need for the improvement of parboiling methods. It is recommended that governmental action in this direction should not be delayed any more. Improved parboiling methods will ensure production of an acceptable article of high nutritive value, will reduce wastage due to broken grains inherent in the milling of raw rice and thus help to conserve the available supplies of rice. It may also be possible to educate an increasingly large proportion of the public to take to parboiled rice in preference to raw rice.

4. The Committee agreed that under the present conditions it is extremely difficult to bring about a rapid improvement in washing and cooking methods of rice in individual homes even with the help of an intense educational programme. An effort should, therefore, be made to supply clean rice to the consumer, for only then will it be possible to avoid or reduce washing which is known to remove 50 to 60 per cent of thiamine contained in it.

The Committee felt that it should be possible to enforce the adoption of steam cooking of rice as a method of choice in feeding establishments such as hotels, communal kitchens, hostels, etc. The Committee, therefore, recommend that Central and Provincial Governments may be moved to explore the possibility of formulating suitable regulations as an experimental measure.

5. The Committee feel that in order to ensure the presence of requisite amounts of thiamine and other vitamins in rice diets, some method of fortification will have to be adopted. This may take the form of addition of the required nutrients to rice or of supplementing rice diet by the supply of vitamins and minerals in tablet form. Before any such recommendation can be put into practice, it will be necessary to have the requisite quantities of vitamins produced in the country. The Committee have been informed that it may be practicable to arrange for the production of necessary vitamins in India in sufficient quantities at an early date. The Committee, therefore, strongly recommend to the Government to take necessary steps for their production.

6. The Committee note that the nutritive value of rice and other cereals is influenced by variety, climate, soil conditions and methods of cultivation. Necessary data in this connection are, however, not available at present. In view of the desirability of the production of superior varieties of rice and other cereals, the Committee recommend that necessary investigations be undertaken immediately to determine the variations found in the nutritive value of the varieties of rice and other cereals, on which a programme of breeding such varieties could be based.

7. The Committee feel that large amount of nutritious food can be made available by paying immediate attention to conserve the fish supplies. A major proportion of these are being lost on account of faulty methods which are capable of improvement even with the present equipment and facilities. If facilities for quick transport, marketing and processing of fish are conceded high priority, the population of the coastal regions of India will be able to considerably augment their food supplies. Developmental plans for deep-sea fishing, refrigeration, adoption of modern processing methods, etc., can meanwhile be implemented. They, however, need not hold up the action in the direction suggested above.

8. The Committee recommend that the production of food yeast should be taken in hand as an immediate measure. Food yeast manufacture has been strongly recommended by the Bhore Committee (Health Survey and Development Committee). The Committee note with pleasure that there is a proposal on the subject before the Nutrition Committee of the Government of West Bengal, which is expected to be taken up for implementation by the Government on the recommendation of that Committee.

9. The Committee understand that a Food Grains Conservation Committee has been instituted under the auspices of the Government of India. It hopes that the actions taken by this Committee will materially assist in prevention of wastage and hence an increase in the available food supplies. The Committee urge that no effort should be spared to save losses of food occurring through faulty methods of storage and distribution in order that avoidable wastages of available food are prevented.

The Committee thanked Dr. Patwardhan for presenting the Baguio Nutrition Committee report and expressed their appreciation of his work on that Committee.

2. TO CONSIDER THE QUESTION OF OIL EXTRACTION BY CONTINUOUS SOLVENT PROCESS.

The Committee felt that they would like to have more information on the following points :—

1. The minimum and/or optimum requirements of fat by cattle, particularly with reference to :—
   - (*a*) Fat intake and its relation to the yield and composition of milk.
   - (*b*) Fat intake and fæcal excretion of fat.
2. The extent to which solvent residues in the oil and the meal constituted a hazard to human and cattle health owing to the consumption of solvent extracted products over prolonged periods.

The Committee recommended that the question be postponed for consideration at their next meeting and that during the intervening period information on the above points may be made available to them.

3. TO CONSIDER THE QUESTION OF COLOURIZATION OF VANASPATI.

The Committee discussed the question at some length and agreed to hold a joint meeting with the Vanaspati Research Advisory Committee of the Council of Scientific and Industrial Research at a later date. Members were requested to submit memoranda on the problem for circulation among members of both the Committees.

4. TO CONSIDER A NOTE PREPARED BY DR. K. MITRA ON LOW BODY TEMPERATURE AND MALNUTRITION.

The Committee were of the opinion that malnutrition *per se* was not known to cause an increase in body temperature. On the other hand, in undernutrition, body temperature tends to be lower than normal.

Regarding the particular observations presented by Dr. Mitra, the Committee felt that as—

(*a*) the temperatures were not taken at comparable times during the day,

(*b*) the blood picture reported in a few individuals indicated the presence of active infective processes and

(*c*) the other data were insufficient,

they were not in a position to express a definite opinion.

5. TO CONSIDER THE SECOND REPORT OF THE SUB-COMMITTEE ON THE ADOPTION OF UNIFORM METHODS FOR THE EVALUATION OF NUTRITIONAL STATUS OF POPULATION GROUPS.

The Committee accepted the report of the Sub-Committee (*Appendix I*) and recommended that it should be widely circulated.

6. TO CONSIDER THE DRAFT NOTE PREPARED BY THE SUB-COMMITTEE CONSISTING OF DR. K. MITRA (CONVENER), DR. RAJAGOPAL, DR. S. BANERJI AND DR. KARUNAKARAN ON THE RESULTS OF DIET SURVEYS.

The Committee considered the minutes of the first meeting of the Sub-Committee (*Appendix II*) and took note of the fact that the draft was not yet ready. Dr. Mitra and his Sub-Committee were requested to prepare the draft in time for consideration at the next meeting of the Nutrition Advisory Committee.

In view of the large amount of work involved and to meet the expenditure involved in preparing cyclostyled copies of the draft report, the Committee recommended that Dr. Mitra may be given the assistance of a clerk on Rs. 80/- p.m. in the scale Rs. 80-5-120-E.B.-8-200-10/2-220 till the 31st December, 1949 and a grant of Rs. 500/- be made available for contingent expenses.

7. TO CONSIDER THE DRAFT OF A POPULAR MONOGRAPH ON WHEAT PREPARED BY DR. K. MITRA, DR. K. P. BASU AND DR. K. RAJAGOPAL.

The Committee noted that the draft of the monograph on wheat was not ready. They requested Dr. Mitra to submit it for consideration at their next meeting in November, 1949.

8. TO CONSIDER THE PROGRAMME OF RESEARCH OF THE NUTRITION RESEARCH UNIT AT THE INDIAN INSTITUTE OF SCIENCE, BANGALORE FOR 1949-50.

The Committee recommended that the Nutrition Research Unit should be under the technical charge of Dr. S. S. De, Assistant Professor of Food Technology at the Indian Institute of Science, Bangalore. They also recommended that Dr. De may, to begin with, restrict his attention to the studies on the amino-acid content of cereals and pulses. The administrative charge of the Unit will rest with the Head of the Biochemistry Department of the Indian Institute of Science. The Committee were pleased to note that Mr. B. N. Banerjee, Acting Professor of Biochemistry, Indian Institute of Science, Bangalore, who attended the meeting of the Nutrition Advisory Committee, assured them of his assistance and co-operation in the work of the Unit.

APPENDIX I.

Second Report of the Sub-Committee on the Adoption of Uniform Methods for the Evaluation of Nutritional Status of Population Groups.

The second meeting of the Sub-Committee was held at the All-India Institute of Hygiene & Public Health, Calcutta on the 6th July, 1949 at 10-45 a.m. The following were present :—

Dr. G. Sankaran. (*Convener*).
Dr. K. Mitra.
Dr. V. N. Patwardhan.
Dr. M. V. Radhakrishna Rao.
Dr. K. Rajagopal. } (*Co-opted members*).

The Sub-Committee considered two draft notes on :—

1. Methods to be used in rapid nutritional surveys.
2. Nutritional surveys supported by laboratory investigations.

The approved drafts of these notes are attached as *Annexures I* and *II*.

Annexure I.

NOTE ON RAPID NUTRITIONAL SURVEYS.

At the last meeting of the Nutrition Advisory Committee of the Indian Research Fund Association, held in Madras on the 1st December, 1948, the Committee had approved of a Nutrition Assessment Schedule to be used ordinarily by the nutrition workers in the country when they carry out nutritional surveys of random samples of population groups. It was, however, felt that whenever it was intended to carry out investigations on large groups of individuals within a short space of time to determine their nutritional status, a less comprehensive examination would be desirable. The present note is, therefore, drawn up for the purpose.

Expressions such as 'deficiency states', 'malnutrition', 'under-nutrition', 'nutritional deficiencies', 'deficiency disease', etc., have been used rather vaguely in scientific literature on nutrition. This has necessarily caused a certain amount of confusion in exactly defining what each of these conditions are likely to imply in terms of the deviation from normal health. Clinical nutrition workers in the West, particularly in the U. S. A., have laid considerable stress on elaborate clinical examination of various parts of the body. It is, however, well known that gross malnutrition or under-nutrition are conditions not ordinarily met with in the comparatively well-fed countries of the West. Consequently, the workers there had to devise an extensive clinical schedule for grading nutritional status. The Committee feels that in India signs of lowered state of nutrition are so apparent even to casual observers that appreciable curtailment in the items of clinical examination is not likely to reduce the value of such findings.

The following tentative classification is, therefore, suggested in order to avoid the confusion in terminology in grading the nutritional states :—

Grade I.—It consists of individuals who appear healthy and do not exhibit evidence of any of the recognized clinical signs of nutritional disorders. It is quite possible, however, that persons suffering from sub-clinical nutritional states discernible by elaborate laboratory examinations, are likely to be included in this group.

Grade II.—In this group are included persons who show mild signs of any one of the specific nutritional disorders.

Grade III.—Persons included in this group show marked clinical signs of lowered state of nutrition.

The necessity for a rapid nutritional survey in times of emergency is fully recognized and at the same time the dangers associated with a very hurried appraisal of nutritional status without proper consideration of those manifestations which are ordinarily associated with lowered state of nutrition are not ignored. Consequently, the approach to the problem must necessarily be through some of the more widely accepted criteria enumerated in the Nutrition Assessment Schedule approved by the Nutrition Advisory Committee and referred to in the opening paragraph. The possibility of the technique of rapid nutritional survey described in this note being revised on the experience of future work in this country is recognized.

The Committee would like to emphasize certain points which may be overlooked in the stress and strain of emergency. The fact should not be forgotten that (*a*) even for a rapid nutritional survey a minimum amount of time has to be devoted and reasonably adequate samples of population groups examined to arrive at a fairly precise appraisal of the nutritional status of individuals in any given area, (*b*) it is essential that the work be entrusted to a medical man specially trained in this technique and (*c*) that the persons who may be detailed on such investigations should be provided with adequate staff and equipment for carrying out the survey (vide *Appendix I*).

The Committee recommends a specific schedule (vide *Appendix II*) for rapid nutritional surveys proposed in this note.

The average figure for weights in different groups of population still maintains its importance in any inquiry in spite of its limitations. It is realized that average weights of any group of individuals for the first time may not yield a reliable index for the assessment of nutritional status of that group in the absence of norms. For successive examinations of the same or similar groups of population the average weight records should indicate the trend in the improvement or otherwise of nutritional status.

The dangers of including any of the complaints, such as lassitude, feeling of early fatigue, anorexia, etc., in the schedule are very great, particularly in areas where the majority of population is illiterate. Under grade II lack of interest in surroundings has been included as one of the items for examination. Here the investigator is to be guided solely by his observations and not by direct questioning of the subject under examination.

No nutrition survey howsoever rapid can be considered as complete unless investigations on dietary habits are also made. It is, therefore, suggested that the Medical Officer-in-Charge of each team should, during the investigations, make careful inquiries about dietary habits of the people examined and append his comments to his report. However, if any particular individual during his physical examination volunteers information on diet which may be different or deserves mention it should be included under the item special remarks. Similarly, history of recent attacks from any of the specific diseases may be entered under the item.

Appendix I.

The team for rapid nutritional surveys should consist of :—

1 Medical officer.
1 Technical Assistant.
1 Attendant.

Such a team can conveniently examine on the average about 50 to 60 persons on each of the working days.

The main equipment and appliances should consist of :—

1 Portable weighing machine.
1 Sahli's hæmoglobinometer.
25 Sahli's tubes.
Necessary chemicals in a box.

Appendix II.

SCHEDULE FOR RAPID NUTRITIONAL SURVEY.

Serial No.
Name
Age
Sex
Place
Weight lb.
Height inches
Hæmoglobin g./100 c.c.

GRADE I

GRADE II

(*a*) Poor musculature
(*b*) Deficient subcutaneous fat
(*c*) Mild anæmia
(*d*) Lack of interest in surroundings
(*e*) Mild signs of not more than one of the specific nutritional disorders or deficiencies mentioned under grade III.

GRADE III

(*a*) Gross muscular wasting
(*b*) Xerosis or pigmentation of conjunctiva
Bitot's spots
Xerosis of cornea
Angular conjunctivitis (bilateral)
(*c*) Marked anæmia
(*d*) Red and/or raw tongue ; glazed tongue — Lips = angular
Stomatitis
Bleeding gums
(*e*) Caries
(*f*) Dry and/or rough skin ; crazy pavement skin
Hyperkeratosis
(*g*) Œdema, dependent or generalized
(*h*) Tenderness of the calf
(*i*) Special remarks (if any).

N. B.—In filling up the schedule a reference is requested to the explanatory note.

a.) Appetite. 0 Normal
1 Poor

Annexure II.

NOTE ON 'NUTRITIONAL SURVEY' SUPPORTED BY LABORATORY INVESTIGATIONS.

The purpose of laboratory investigations in nutrition surveys should be considered from two points of view ;—

(*i*) The laboratory investigations should supplement the information obtained by clinical appraisal methods to enable the nutritional assessment being made on a wider basis of clinical and biochemical findings, or to enable the detection of sub-clinical deficiency states from the biochemical findings in cases with no clinical manifestations.

(*ii*) These investigations should provide a continuous flow of accurate information obtained by observations on fairly large numbers in different parts of the country, the results of which could be utilized—

(*a*) for the establishment of normal values and their range of variation,

(*b*) to provide material for possible improvement of methods, and

(*c*) to revise when necessary, in the light of information obtained, the table of requirements of essential nutrients.

Unless both these objectives are kept in view it is likely that the scientific value of the investigations will be fairly limited. In this report, therefore, both these objectives have been kept in the forefront and hence it is suggested that the laboratory investigations shown in *Annexure III* should be carried out in support of the clinical appraisal made according to the Nutritional Assessment Schedule recommended by the Nutrition Advisory Committee at its meeting held in Madras in December 1948.

The present state of knowledge does not permit drawing any effective conclusions from the results obtained by the tests enumerated in the table. There is a good deal of disagreement amongst various workers on the normal values, the range of variations and interpretation of values thus obtained. The Committee is of the opinion that much more information on all the above aspects will have to be collected before workers in this country can safely draw any conclusions about the effectiveness of biochemical methods in determining the state of sub-clinical nutritional deficiencies. It is felt that unless such work is done in different laboratories, it will not be possible to arrive at any conclusion within a reasonably short period.

In addition to the various investigations mentioned in the table, the following investigations may also be undertaken wherever possible in order to provide further information on some of the points which are likely to be of importance in determining the nutritional status :—

1. Intestinal synthesis of B-group of vitamins and its influence on their excretion in urine.

2. Saturation tests.

There is great divergence of opinion on the significance to be attached to the percentage of test dose excreted and its relation to the state of saturation. It is possible that different interpretations placed on the responses elicited in the load tests may be responsible for the varying figures given for the requirements of some of the vitamins.

3. Vitamin A and carotene level in blood.

The normal range and the relation which these blood constituents have with functional disturbances such as dark-adaptation.

4. The relationship between blood pyruvic acid and B. B. S. and thiamine excretion in urine.

5. The comparison of values of thiamine, riboflavin, niacin and ascorbic acid obtained by the study of one hour fasting sample of urine and determinations made in 24-hour collections.

In the determination of nutritional status with respect to a particular vitamin the assay is carried out usually with a 24-hour collection of urine. This involves considerable inconvenience both to the worker and the subject. The possibility of utilizing the data collected on a single one-hour sample of urine of a fasting subject as indicative of this nutritional state is to be explored. If this approach gives fruitful results it will be eminently practicable.

6. Bone development and growth.

There is no information obtained by *x*-ray studies on the normal development of bone in Indian children. The Committee feels that, since the effects of malnutrition are seen in the retardation of bone growth, later appearance of ossification centres and the union of the epiphyses with the shaft, some work is therefore essential in the normal and malnourished infants and children.

Annexure III.

RECOMMENDED LABORATORY TESTS.

Particulars.	Methods in general use.	Methods recommended and remarks.
I. BLOOD :		
(*i*) Hæmoglobin	Based on (*i*) oxyhæmoglobin, (*ii*) carboxy hæmoglobin, (*iii*) cyanmethæmoglobin, (*iv*) acid hæmatin, (*v*) alkali hæmatin, (*vi*) cyanhæmatin and (*vii*) iron content.	Acid hæmatin. Whichever method is used, the instrument should be standardized against van Slykes' gasometric method or iron estimation according to Wang's method.
(*ii*) Red cell count		Pipettes and chambers should be standardized before use.
(*iii*) Packed cell volume		Wintrobe's tubes should be used.
(*iv*) Blood smear examination		Leishman's stain should be used.
(*v*) Serum, total protein	(*a*) Micro-Kjeldahl, (*b*) specific gravity method : $CuSO_4$, (*c*) Biuret	$CuSO_4$ method (Phillips and van Slyke).
Albumin : Globin	Howe's fractionation method	Howe's method.
(*vi*) Ascorbic acid in whole blood	(*a*) Roe and Kuether's method (*b*) Abt and Farmer's method	Abt and Farmer's method.
(*vii*) Serum alkaline phosphatase	(*a*) Bodansky, (*b*) King and Armstrong	King and Armstrong's method.
(*viii*) Pyruvic acid	2:4–di-nitro-phenyl-hydrozone methods	Bueding and Wortis' method.

II. Urine : (24-hour sample)		
(*i*) Volume (*ii*) Specific gravity (*iii*) Sugar, qualitative test for (*iv*) Albumin, qualitative test for	Approved methods	Approved methods.
(*v*) Creatinine	Folin's method	Folin's method.
(*vi*) Thiamine	(*a*) Fluorimetric, (*b*) microbiological	Thiochrome method of Wang and Harris as modified by Mukundan and Ramasastri.
(*vii*) Riboflavin	,, ,,	Fluorimetric method of B. Sure.
(*viii*) Niacin	(*a*) Colorimetric, (*b*) microbiological	Swaminathan's colorimetric method.
(*ix*) Ascorbic acid in whole blood	Methods using (*a*) 2 : 6-di-chlorophenol-indophenol dye and (*b*) 2 : 4-di-nitro-phenyl-hydrazine.	Titrimetric method using 2 : 6-di-chloro-phenol-indophenol. Harris and Abbasy.
III. Fæces :		
(*i*) Ova (*ii*) Cysts (*iii*) Parasites	Approved methods	Approved methods.
IV. Saturation tests :		
(*i*) Thiamine (*ii*) Riboflavin (*iii*) Niacin (*iv*) Ascorbic acid		

References.

Abt and Farmer (1926) .. *Proc. Soc. Exper. Biol. Med.*, **34**, p. 146.

Bodansky (1932) .. *Jour. Biol. Chem.*, **99**, p. 197.

Idem. (1933) .. .. *Ibid.*, **101**, p. 93.

Bueding and Wortis (1940) .. *Ibid.*, **133**, p. 585.

Folin (1947) .. .. Quoted from Hawk, Oser and Summerson (1947).

Harris and Abbasy (1937) .. *Lancet*, **ii,** p. 1429.

Hawk, Oser and Summerson (1947) .. 'Practical Physiological Chemistry'.

Howe (1921) .. .. *Jour. Biol. Chem.*, **49**, p. 109.

King and Armstrong (1934) .. *Canad. Med. Assoc. Jour.*, **31**, p. 376.

King *et al.* (1948) .. .. *Lancet*, **255**, p. 563.

Kingsley (1942) .. .. *Jour. Lab. Clin. Med.*, **27**, p. 840.

Mukundan and Ramasastri (1948) *Ind. Jour. Med. Res.*, **36**, p. 405.

Phillips, van Slyke *et al.* (1947) Quoted from Hawk, Oser and Summerson (1947).

Roe and Kuether (1943) .. *Jour. Biol. Chem.*, **147**, p 399.

Sure (1947) .. .. *Jour. Nutr.*, **27**, p. 447.

Swaminathan (1946) .. *Ann. Biochem. Exper. Med.*, **2**, p. 75.

Wang and Harris (1943) .. *Brit. Med. Jour.*, **2**, p. 451.

APPENDIX II.

Minutes of the First Meeting of the Sub-Committee on Diet Surveys.

The Sub-Committee appointed by the Nutrition Advisory Committee of the Indian Research Fund Association to prepare a draft note on the results of diet surveys met at the All-India Institute of Hygiene & Public Health, Calcutta on the 6th July, 1949. The following members were present :—

1. Dr. K. Mitra (*Convener*).
2. Dr. K. Rajagopal.
3. Dr. S. Banerjee.

Drs. M. V. Radhakrishna Rao and P. C. Sen attended by invitation.

Records of dietary surveys carried out in the different Provinces and States in India up to the year 1947 had been collected and the crude data were presented before the members of the Sub-Committee. The number of surveys carried out is given below :—

Assam	4
Baroda State	1
Bengal	11
Bhopal State	1
Bihar	49
Bombay	394
C.P. and Berar	14
Cochin State	1
Hyderabad State	1
Indore State	100
Madras	157
Mysore State	2
Orissa	7
Trivandrum	6
Total	748

The Sub-Committee were informed that for various unavoidable reasons no data from Bengal could be collected except the one pertaining to the special survey carried out at Singur by the All-India Institute of Hygiene & Public Health, Calcutta. The Committee were aware of the fact that a large number of surveys had been carried out in Bengal some years ago. The data still remained to be tabulated.

The Sub-Committee discussed how to improve upon the 'Note on the Results of Diet Surveys' compiled by Dr. Aykroyd and published by the Indian Research Fund Association in 1947.

The Sub-Committee were fully alive to the fact that if individual records of all dietary surveys carried out in India be compiled in one report, it was apt to be a voluminous one but at the same time it was felt that as the sampling of surveys in the different Provinces and States were made amongst different economic, social, occupational and communal groups it would be difficult to pick and chose representative samples from these records even from one Province. Further, it was felt that by compilation of all the records in tabular forms these data could be put into use for future studies on the subject by workers in different parts of the country. There was a difference of opinion, however, on the issue whether the data pertaining to each individual survey should be presented in terms of the different classes of foodstuffs, such as cereals, pulses, vegetables, etc., or the values for physiological nutrients should also be expressed. This was an issue the Sub-Committee felt unable to decide upon and it was agreed that the Nutrition Advisory Committee be approached to give its directive on this important issue.

The Sub-Committee also felt that there is sufficient material available to enlarge the scope of the explanatory note and make it more informative regarding the trend of dietary habits existing in the different Provinces and their respective bias for different classes of foodstuffs. It was agreed that a draft explanatory note on the above lines be prepared and circulated to the members of the Sub-Committee before its next meeting.

The Sub-Committee were of the opinion that as there was no planned development of dietary survey work in India and no directives were issued except a short note entitled 'Note on the Statistical Control of Diet Surveys' published by the Nutrition Advisory Committee in June 1944, no uniform method was followed for the collection of data and the presentation of the same in published and unpublished reports. Consequently, it was strongly felt that the matter may be taken up by the Nutrition Advisory Committee and the latter may appoint a Sub-Committee to examine this question and suggest a uniform schedule of inquiry for future diet surveys and the presentation of data.

REPORT OF THE 19TH MEETING OF THE NUTRITION ADVISORY COMMITTEE.

Held in the Central Secretariat, North Block, New Delhi, on the 22nd and 23rd November, 1949.

COMPOSITION OF THE COMMITTEE.

MEMBERS PRESENT.

Dr. G. Sankaran. (*Chairman*).
Dr. S. Banerji.
Dr. K. P. Basu.
Professor M. Damodaran.
Dr. B. C. Guha.
Dr. K. Mitra.
Dr. M. V. Radhakrishna Rao.
Professor V. Subrahmanyan.
Dr. V. N. Patwardhan. (*Secretary*).

Dr. B. B. Dikshit was unable to attend.

VISITORS.

Dr. R. K. Anderson.
Dr. A. C. Banerjea.
Dr. Balmokand.
Lieut.-Colonel Barkat Narain.
Dr. (Miss) S. Chaudhuri.
Brevet-Colonel R. N. Chopra.
Group-Captain D. N. Chakravarti.
Dr. M. B. Daver.
Dr. S. S. De.
Lieut.-Colonel A. N. Duggal.
Dr. P. V. Gharpure.
Dr. Gwash Lal.
Dr. K. K. Govil.
Dr. A. Gilroy.
Dr. C. R. Das Gupta.
Dr. (Miss) R. Karnad.
Dr. V. R. Khanolkar.
Dr. D. V. Karmarkar.
Major S. L. Kalra.
Dr. R. B. Lal.

Dr. S. B. Lal.
Lieut.-Colonel C. K. Lakshmanan.
Dr. A. R. Mehta.
Dr. Daulat Ram Mehta.
Leiut.-General K. S. Master.
Dr. A. C. Munro.
Dr. K. K. P. Narasinga Rao.
Colonel S. Narain.
Mr. S. S. Pillai.
Dr. D. M. Roy.
Dr. K. Rajagopal.
Dr. R. Subramaniam.
Dr. Muktha Sen.
Mr. S. H. Turner.
Lieut.-Colonel O. P. Verma
Dr. N. Veeraraghavan.
Dr. K. V. Venkatraman.
Dr. Cicely D. Williams.

Dr. C. G. Pandit, Secretary, Indian Research Fund Association, also attended the meeting.

In making the following recommendations, the Committee scrutinized the budgets very carefully and in view of the stringent financial situation suggested certain economies in the budgets proposed for the old and new inquiries to which the members unanimously agreed. Hence, no system of priorities was adopted in recommending the continuation of old and adoption of new inquiries.

1. TO REVIEW WORK IN PROGRESS AND CONSIDER THE QUESTION OF CONTINUATION OR OTHERWISE OF EXISTING INQUIRIES.

(A) The Committee recommended the continuation of the following inquiries :—

(*i*) Nutrition researches under Dr. V. N. Patwardhan at the Nutrition Research Laboratories, Indian Research Fund Association, Coonoor.

(*ii*) Nutrition research unit under Dr. B. C. Guha at the University College of Science & Technology, Calcutta.

(*iii*) Inquiry into metabolism of vitamin A (excretion of vitamin A in urine) under Dr. K. Rajagopal, Associate Professor of Nutrition, All-India Institute of Hygiene & Public Health, Calcutta.

(*iv*) Inquiry on physiological effect of dietary protein under Dr. M. Damodaran at the National Chemical Laboratory, Poona.

(*v*) Inquiry into the nutritive value of diets prescribed for hospital patients in India under Dr. K. Mitra, Adviser in Nutrition, Directorate-General of Health Services, Government of India, New Delhi.

(*vi*) Inquiry on the studies on the biosynthesis of nicotinic acid under Dr. S. Banerji, Head of the Department of Physiology, Presidency College, Calcutta.

Dr. Banerji did not wish to proceed with this inquiry as he intended to carry on this problem in a larger investigation submitted for consideration to the Nutrition Advisory Committee under new proposals. The Committee, however, felt that Dr. Banerji had obtained promising results in this work and that he should continue with it. The Committee requested Dr. Banerji not to press for his other proposals before the Indian Research Fund Association and to submit a new budget for continuation of the present work. Dr. Banerji agreed to withdraw his other proposals.

(*vii*) Nutrition research unit under Dr. S. S. De at the Indian Institute of Science, Bangalore.

The Committee was glad to note that Dr. De was likely to get equipment for work on radio-isotopes. The Committee agreed to consider his request for assistance on metabolism of iron next year if during the interval the equipment had been installed at Bangalore and found working satisfactorily. In the meantime, the Committee requested Dr. De to continue with his work on amino-acid composition of cereals.

(*viii*) Inquiry into the effects of malnutrition on the physique and more particularly on the blood picture including hæmoglobin and plasma-protein concentration as also the treatment thereof with high-protein diets, protein hydrolysates, etc., under Professor (Miss) S. Chaudhuri and Dr. K. Mitra at the Lady Hardinge Medical College, New Delhi.

The Committee recommended the upgrading of Dr. (Miss) Sushila Shyam Swarup to the grade of Assistant Research Officer on the starting salary of Rs. 275/- p.m. in the scale Rs. 275-25-500-EB-30-590. The Committee also recommended that she should be deputed by the Indian Research Fund Association to Calcutta School of Tropical Medicine for a period of one month for training in latest technique of biochemical investigations on blood. Extra provision in the budget for this purpose was recommended.

(*ix*) Nutrition research unit under Dr. R. G. Chitre at the Seth G. S. Medical College, Bombay.

The Committee did not feel satisfied with the conduct of inquiry at this unit during the last two years. It felt that Dr. Chitre had not followed the recommendations made by the Nutrition Advisory Committee at its 17th Meeting. Under the circumstances, the Committee was of the opinion that the objects with which the Indian Research Fund Association established this unit were no longer being adequately fulfilled. However, as Dr. Chitre was abroad and likely to return in September 1950, the Committee wished to give him a chance to state his case next year, but it was of the opinion that as there was no guarantee of effective direction during his absence, the staff at his unit should be reduced to the following:—

One assistant research officer.

One senior laboratory assistant.

One laboratory attendant.

Provision for the remainder of the staff should be made, if possible, on other new inquiries recommended by the Committee.

(B) The following inquiry terminates at the end of the financial year :—

Inquiry on the preparation of anti-rachitic substances under Professor B. V. Bhide at the Maharaja Pratap Sinh Chemical Laboratory, S. P. College, Poona.

As the Committee was informed that Professor Bhide would like to continue his work on the problem without further assistance from the Indian Research Fund Association, it recommended that he should be allowed the use of the high-pressure hydrogenation apparatus ordered for him last year by the Indian Research Fund Association. The apparatus should, however, be transferred under the charge of Dr. M. Damodaran, Officer in-Charge of an inquiry under the Indian Research Fund Association. If any worker under the Indian Research Fund Association needed the use of the apparatus, the Secretary, I.R.F.A. would consider the question on receipt of such information.

2. TO CONSIDER NEW PROPOSALS.

(A) The Committee recommended the following new inquiries :

(*i*) Microbiological investigations in the B-group of vitamins under Dr. A. Sreenivasan at the Department of Chemical Technology, University of Bombay, Bombay.

(*ii*) Inquiry on the enzymic role of inositol under Dr. P. S. Sarma at the University Biochemical Laboratory, University of Madras, Madras.

(*iii*) Inquiry to study the digestion, evacuation time and gastric response to various Indian food preparations under Dr. J. D. Pathak at the Topiwala National Medical College, Bombay.

(*iv*) Inquiry on the nutritive value of Indian dairy products under Dr. K. P. Basu at the Indian Dairy Research Institute, Bangalore.

(*v*) Inquiry to assess the value of supplementing rice diet with de-fatted groundnut cake flour fed to children under Dr. S. B. Lal, Officer in-Charge, Nutrition Scheme, Bihar.

The Committee recommended that Dr. K. Mitra might be requested to make himself available for consultation and advice when needed.

(B) The following inquiries were not recommended :

(*i*) Inquiry on the production of hydrolysed protein from fish flesh under the Assistant Director of Fisheries (Technology), Orissa, Cuttack.

(*ii*) Inquiry on the actual difference between the nutritive value of raw foods and when they are ready for ingestion, etc., under Mr. B. Banerjee at the Assam Medical College, Dibrugarh.

The Committee recommended that Dr. Banerjee should submit a proposal next year if during the interval his department has been equipped suitably for the conduct of such investigation. The Committee will give favourable consideration to such a proposal.

(C) The following inquiries were withdrawn by the sponsors:

(*i*) Studies on the nutritive value of different strains of pulses and cereals grown in India and the effect of germination on the same under Dr. S. Banerjee at the Presidency College, Calcutta.

(*ii*) Inquiry to assess the biological value of nitrogen balance method of de-fatted groundnut cake flour fed along with wheat, rice and dal to human volunteers under Dr. S. B. Lal, Officer in-Charge, Nutrition Scheme, Bihar.

3. TO CONSIDER THE PROGRESS OF NUTRITION WORK IN PROVINCES AND STATES.

The Provincial Nutrition Officers who were present gave an account of the nutrition work carried out in various Provinces. From some Provinces only reports were received but the Nutrition Officers concerned were not in attendance to present the reports. These reports were taken as read. The following is the general outline of the work carried out in Provinces. It may be mentioned that in general the nutrition work in various Provinces had been carried out along certain well defined lines, such as diet and nutrition surveys, ameliorative measures, propaganda and educative activities and advisory work. In only a few Provinces laboratory work was possible.

Bihar.

Diet surveys were carried out in two districts on 300 families of which 200 were aboriginal families. The results confirmed the previous findings from other rural areas in the Province that the diets were deficient in protective foodstuffs. Nutritional surveys of children belonging to these families were carried out in accordance with the Nutritional Assessment Schedule recommended by the Nutrition Advisory Committee of the Indian Research Fund Association. The material is being evaluated. The Nutrition Section took part in certain propaganda and education activities. It has also proposed the commencement of certain ameliorative schemes, which are under consideration of the Government.

Bombay.

The work of the Nutrition Section was devoted, to a very large extent, to studying the conditions in relief camps for displaced persons. The diet scales were examined and revised. Conditions arising out of nutritional disorders were examined and treated. Rapid Nutritional Surveys according to the Schedule approved by the Nutrition Advisory Committee of the Indian Research Fund Association have been carried out on 550 children. The Section also supervises the 'Square Meal' Canteen run by the Government for the staff of the Bombay Secretariat. The Section advises the Government on diet scales for hospital patients and prisoners. It has kept in touch with other activities of the Bombay Government in regard to the increased food production.

Central Provinces & Berar.

Diet surveys and nutrition surveys were undertaken by the Nutrition Section and the Nutrition Officers also initiated activities with regard to education of the public by talks, articles and film shows.

East Punjab.

Nutrition surveys were carried out among displaced persons in the Kurukshetra camp. From the sample surveys it was found that night blindness, phrynoderma, Bitot's spot, cheilosis, etc., were prevalent. The diets in general were found unsatisfactory. Iodized salt had been distributed to consumers in Kangra valley with a view to testing its effect on the incidence of endemic goitre.

Hyderabad State.

Diet surveys were carried out in urban and rural areas on families of industrial workers. A total of 136 families were surveyed. Diets of these families were found to be particularly deficient in protective foodstuffs. In most cases calorie intake fell far short of the recommended allowance. A major portion of the families were investigated from the standpoint of nutritional status. High incidence of xerophthalmia, angular stomatitis and glossitis was found.

School children numbering 3,103 were examined for deficiency diseases and deficiency of Vitamins A, B complex and D was found fairly widespread.

Madras.

Diet surveys were carried out on 642 families representing about 3,400 individuals in 12 districts of Madras Province. The surveys confirmed more or less the findings made in earlier years, with regard to the deficiency in calories in several areas, deficiency of protective foodstuffs and general imbalance of diets. Nutrition surveys of 6,100 children were carried out according to the Schedule recommended by the Nutrition Advisory Committee. The material is under investigation although 1,900 children showed evidence of deficiency state. The Section is continuing its investigation on physical measures for the purpose of preparing standards for comparison with regard to heights and weights of children.

Free midday meal to school children and free milk distribution was continued under the auspices of the Corporation of Madras and the Government. There is, however, some danger that owing to financial stringency these schemes may be curtailed, if not altogether abolished.

Orissa.

The report from Orissa mentions that the Government could not carry out any work on nutrition for want of trained personnel. The Committee was informed that one Nutrition Officer and two Health Inspectors were trained at the Nutrition Research Laboratories, Coonoor in 1947. In view of this the statement does not appear to be correct.

United Provinces.

The Committee took note of the fact that the United Provinces have now established a Nutrition Section in the Public Health Directorate. Although the work reported by the Province was very small, it hoped that next year it would be able to report further progress in the work.

4. TO CONSIDER NUTRITIONAL SURVEY REPORTS SUBMITTED BY PROVINCIAL NUTRITION OFFICERS, THE FINDINGS OF WHICH HAVE BEEN RECORDED ACCORDING TO THE NUTRITIONAL ASSESSMENT SCHEDULES RECOMMENDED BY THE NUTRITION ADVISORY COMMITTEE (PARA 7 OF THE REPORT OF THE 17th MEETING OF THE NUTRITION ADVISORY COMMITTEE, DECEMBER 1948).

The Committee felt that it did not have sufficient material adequately prepared to enable it to discuss the problem. They, therefore, recommended that the question be considered at its next meeting and requested the Secretary, Nutrition Advisory Committee to obtain the necessary information and to place it for consideration by the Sub-Committee on Evaluation of Nutritional Status; the Sub-Committee was re-constituted as follows :—

1. Dr. G. Sankaran (*Convener*).
2. Dr. K. Mitra.
3. Dr. M. V. Radhakrishna Rao.
4. Dr. K. Rajagopal.
5. Dr. V. N. Patwardhan.

5. TO CONSIDER THE QUESTION OF PUBLICATION OF A REVIEW OF NUTRITION WORK DONE IN INDIA AND THE INFLUENCE WHICH IT HAD UPON THE HEALTH OF THE PEOPLE OF THE COUNTRY.

The Committee appointed the following Sub-Committee to prepare a draft of the review which might be considered at its next annual meeting :—

1. Dr. M. V. Radhakrishna Rao.
2. Dr. K. Mitra.
3. Dr. V. Subrahmanyan.
4. Dr. V. N. Patwardhan (*Convener* and *Secretary*).

6. TO CONSIDER THE FOLLOWING PAPERS ON THE PRODUCTION AND POPULARIZATION OF SOYA-BEAN MILK.

(*a*) Letter No. F.6-9/49-PHII, dated the 28th September, 1949 from the Secretary, Government of India, Ministry of Health, New Delhi.

(*b*) Letter No. FTRI/2212/63, dated the 20th September, 1949 from Professor V. Subrahmanyan, Planning Officer, Central Food Technological Research Institute, Mysore.

The Committee postponed consideration of the subject to its next meeting, to which the Ministry of Agriculture should be requested to depute technical experts. In the meantime information on some of the relevant points should be obtained and papers circulated.

7. TO CONSIDER THE DRAFT NOTE PREPARED BY THE SUB-COMMITTEE CONSISTING OF DR. K. MITRA (CONVENER), DR. K. RAJAGOPAL, DR. S. BANERJEE AND DR. M. V. RADHAKRISHNA RAO ON THE RESULTS OF DIET SURVEYS (PARA 6 OF THE REPORT OF THE 18th MEETING OF THE NUTRITION ADVISORY COMMITTEE, JULY 1949).

The Committee took note of the fact that Dr. K. Mitra, Convener of the Sub-Committee, had prepared and circulated the report. As the draft reached the members fairly late, there was not sufficient time for its consideration. The Committee, therefore, recommended that the members should intimate their comments on the draft to the Secretary, Nutrition Advisory Committee, so as to reach him not later than the 31st December, 1949, who would pass them on to Dr. K. Mitra. Dr. Mitra would consult the other members of the Sub-Committee and the agreed opinion of the Sub-Committee on this subject would be forwarded to the Secretary of the Nutrition Advisory Committee.

The Committee authorized the Chairman and the Secretary of the Nutrition Advisory Committee to incorporate such agreed suggestions and to proceed with the printing of the draft copy which may be circulated to the members of the Committee for consideration at its next meeting in galley-proof stage.

The Committee agreed to the suggestion made by the Sub-Committee that the staff at present with Dr. Mitra should continue till the 31st March, 1950.

8. TO CONSIDER THE DRAFT OF A POPULAR MONOGRAPH ON WHEAT PREPARED BY DR. K. MITRA, DR. K. P. BASU AND DR. K. RAJAGOPAL (PARA 7 OF THE REPORT OF THE 17th MEETING OF THE NUTRITION ADVISORY COMMITTEE, JULY 1949).

The Committee postponed the consideration of the subject to its next meeting as the draft of the popular monograph on wheat was not ready. The Committee took note of the assurance given by Mr. K. K. P. Narasinga Rao, a member of the staff of the Nutrition Division of the F. A. O., who was present at the meeting, that relevant material collected by the F. A. O. would be forwarded to Dr. Mitra at an early date.

9. TO CONSIDER THE QUESTION OF INVESTIGATION OF DEVELOPING A RAPID AND RELIABLE METHOD OF THIAMINE ESTIMATION IN RICE FOR USE IN MILLS AND LABORATORIES [PARA I(1) OF THE REPROT OF THE 18th MEETING OF THE NUTRITION ADVISORY COMMITTEE, JULY 1949].

The Committee recommended that various laboratories and institutions in India interested in the investigation on methods of estimation of thiamine in cereals should present their reports at the next meeting of the Nutrition Advisory Committee for its consideration.

10. TO CONSIDER THE QUESTION OF OIL EXTRACTION BY CONTINUOUS SOLVENT PROCESS (PARA 2 OF THE REPORT OF THE 18TH MEETING OF THE NUTRITION ADVISORY COMMITTEE, JULY 1949).

The Committee recommended that as the problem was of importance from the standpoint of human and animal nutrition, it would be desirable to have a joint meeting with the Animal Nutrition Committee of the Indian Council of Agricultural Research or with animal nutrition experts deputed for the purpose from the Animal Husbandry Wing of the Board of Agriculture with the Government of India.

11. The following resolution proposed by Dr. B. C. Guha, Head of the Department of Applied Chemistry, University College of Science & Technology, Calcutta and seconded by Dr. V. N. Patwardhan, Director, Nutrition Research Laboratories, Coonoor, was unanimously adopted by the Committee :—

" This Committee has taken into consideration the valuable work done by the Nutrition Officers of the various Provinces and it feels that adequate information exists to enable the Governments to plan and carry out ameliorative schemes of short and long term character. The Committee is of the opinion that the Provincial Governments should now take early steps in this direction.

In order that such schemes should be fruitful in improving the health of the people and prove of lasting benefit, the Committee recommends that the Nutrition Sections of the Public Health Departments should be adequately strengthened and provided with the laboratory facilities and equipment, staff and funds to enable them to take effective parts in the Governments' programmes.

This Committee also urges on the Nutrition Officers to take the initiative in formulating concrete measures with regard to the improvement of the nutrition level in their respective Provinces and make ceaseless efforts to have these implemented with the active assistance of the Governments, the municipalities, school authorities and other sections of the enlightened public."

12. Dr. M. Damodaran, Head of the Division of Biochemistry, National Chemical Laboratory, Poona had placed for consideration of the Committee a resolution on the desirability of investigations on the relation between human nutrition and milling of rice in all its aspects. The Committee had discussed this question at its 18th meeting held in Calcutta on the 7th and 8th July, 1949 while discussing the report of the Nutrition Committee (South East Asia Region) of the Food and Agricultural Organization of the United Nations held in Baguio, Philippines, from 23rd to 28th February, 1948 and had made certain recommendations as mentioned on this subject. Any further specific work to be pursued in the matter might be considered at the next meeting.

LEPROSY.

1. Leprosy inquiry under Dr. Dharmendra at the School of Tropical Medicine, Calcutta.

During the year the work of the inquiry has been concerned mainly with the therapeutic studies in leprosy, though some work has also been done on the histopathology, and the study of nose and throat lesions in leprosy. The therapeutic studies have mostly been in connection with the investigation on the use of sulphetrone by intramuscular injections, though the oral administration of sulphetrone and diasone has been continued. Another drug studied has been the para-amino salicylic acid ; this study has been on a very limited scale on account of the limited supply of the drug. An account of the investigations on parenteral administration of sulphetrone, and on the use of para-amino salicylic acid in the treatment of leprosy is given below.

INTRAMUSCULAR ADMINISTRATION OF SULPHETRONE.

Reasons for the study.—During the past few years work has been carried on under this inquiry on the therapeutic value of the sulphone drugs in leprosy. In this study were included promin (given intravenously), and diasone and sulphetrone (both given orally). Promin was found to be more toxic than the other two, and later the study was concentrated on diasone and sulphetrone.

These drugs have been found very effective in the treatment of leprosy, but they are very costly and beyond the means of most patients in this country. By the oral route, a large portion of the drug passes out unabsorbed and is thus wasted ; moreover, it was found that after the oral administration for a few months, the same dose gives rise to a lower blood concentration than in the beginning of the treatment. It was considered that the parenteral administration, by eliminating the question of defective absorption would be more effective, and more economical, thereby bringing down the cost of treatment. This question has been investigated.

Objects of the study.—The objects of the investigation have been to study the pharmacological and therapeutic aspects of the parenteral administration of sulphetrone, in order to find out a suitable preparation, dose and interval for the intramuscular injection.

Another object in undertaking the work was to investigate the advantages, if any, of the combined treatment with the sulphones and hydnocarpus oil. Both hydnocarpus oil and the sulphones are known to be useful in the treatment of leprosy, but both have their limitations. It is possible that the combined treatment with the two might give better results than either alone.

The material for the study.—

The patients : The study has been made in advanced lepromatous cases of leprosy, bacteriologically positive and lepromin-negative, in-patients of the Leprosy Hospital, Gobra. Eighty patients have been included in the study. Many of them were suffering from chronic ulcers, eye complications, and were subject to frequent reactions.

The preparations of sulphetrone : The fine levigated powder of sulphetrone used in this study has been kindly supplied by the manufacturers, Messrs. Burroughs Wellcome & Co. Using this powder, six different preparations were made in order to find out the most suitable preparation from the point of view of producing the highest blood concentration and maintaining it longest. The following are the six preparations, all containing 30 per cent of sulphetrone powder :—

(*i*) Aqueous solution.
(*ii*) Suspensions in hydnocarpus oil without wax.
(*iii*) Suspensions in hydnocarpus oil with 1 per cent wax.
(*iv*) Suspension in arachis oil without wax.
(*v*) Suspension in arachis oil and with 4 per cent wax.
(*vi*) Emulsion in hydnocarpus oil and water.

Aqueous solution was included since the powder is highly soluble in water, and as such a watery solution claimed first attention. Oily suspensions were included to see if they had any 'depot' effect, releasing the sulphetrone gradually and over prolonged periods ; hydnocarpus oil was used in order to get the combined therapeutic effect of sulphones and the oil. The addition of bees wax to these suspensions was made with the idea of further enhancing this 'depot' effect (as the hydnocarpus oil is very viscid, only 1 per cent wax was added to it, while 4 per cent wax was added to the arachis oil suspension). The emulsion with hydnocarpus oil was made in order to produce an easily injectable preparation of sulphetrone and hydnocarpus oil.

Before these preparations were actually used in patients, preliminary investigations were made to ensure their suitability and freedom from local pain and irritation. These preliminary investigations included (*i*) the testing of their acid values, (*ii*) dermonecrosis tests in guinea pigs after intradermal injections, and (*iii*) tissue necrosis tests in guinea pigs after intramuscular injections.

The methods of work.—The investigations have included studies of the following subjects :—

(*i*) Blood concentrations and maintenance after injections of varying doses of the six different preparations.
(*ii*) Excretion of the drug in urine.
(*iii*) Concentration in the skin as compared to the blood concentration.
(*iv*) Liver function tests for detecting any liver damage.
(*v*) Blood examination to study the degree of anæmia produced.
(*vi*) Repeated bacteriological examinations to study the bacteriological improvement.
(*vii*) Frequent clinical examinations to detect any clinical progress or complication.

In the study of the pharmacological aspects a very large number of tests has been done for estimating the concentration of the drug in blood and urine, i.e. about 4,000 blood estimations and about 2,000 urine estimations.

BLOOD CONCENTRATIONS OF SULPHETRONE.

The smallest dose producing detectable blood concentration.—Detectable amounts of the drug in blood were produced by a dose of 0·03 g. of sulphetrone, but the concentration was too low to be estimated by colorimeter. With this dose the concentration in the urine at two hours was from 5 mg. to 10 mg. per cent, and it was still present at 24 hours though the concentration was below 2 mg. per cent.

With a dose of 0·05 g. a concentration of 0·5 mg. per cent was found in blood after two hours ; 12 hours after the injection, the drug could be detected in only half the cases and the concentration was lower ; at the end of 24 hours the blood was free from the drug. With this dose, the concentration in urine is much higher, and it can be detected for 48 to 72 hours after the injection though in low concentration.

Earliest appearance in blood.—It has been found that in case of both the oily suspension and the watery solution, the drug appears in estimable concentrations within five minutes after the injection. The concentration continues to rise up to two hours and thereafter begins to fall. With the same dose the concentration in case of watery solution is higher than in case of the oily suspension.

Maintenance of the blood level.—The highest concentration is reached two hours after the injection, thereafter the concentration begins to fall and at 12 hours it is appreciably below the two-hour level. Investigations were made to find out for how long the drug was present in estimable concentrations in the blood. The matter was first studied in detail with reference to the aqueous solution of the drug, and later comparisons were made with other preparations.

Aqueous solution.—With the aqueous solution the following results were obtained :—

With a dose of 0·1 g. to 0·2 g. the average initial level is below 2 mg. per cent ; by 12 hours the level falls to below 1 mg. per cent and at 24 hours there is practically no or only traces of the drug in the blood.

With a dose of 0·3 g. the average initial level is about 3 mg. per cent ; by 12 hours it falls to 1 mg. per cent, and by 24 hours to about 0·5 mg. per cent, and the blood is free within 48 hours.

With doses from 0·5 g. to 1·5 g. the average initial concentration is between 4 mg. and 6 mg. per cent, by 12 hours it falls to 2 mg. to 3 mg. per cent ; by 24 hours it is between 1 mg. and 1·5 mg. per cent, and by the end of 48 hours it falls to below 1 mg. per cent.

With a dose of 2 g. the average initial concentration is about 9 mg. per cent which falls to about 4·5 g. per cent by 12 hours, and to 3·5 mg. per cent at 24 hours, and is about 1·5 mg. per cent at 48 hours. At the end of 72 hours, the concentration is very low, varying from 0·5 mg. per cent to traces.

It may, therefore, be concluded that in case of the watery solution, the blood is practically free from the drug at the end of 24 hours after a dose of 0·1 to 0·3 mg. ; at the end of 48 hours after a dose of 0·6 g. to 1·5 g. ; and at the end of 72 hours after a dose of 2 g.

Other preparations.—A study was then made to compare the findings in watery solution with those in the other preparations. It was found that the preparations containing water (aqueous solution and the hydnocarpus emulsion) give rise, dose for dose, to a little higher blood concentrations than the oily suspensions, but that the drug is maintained in the blood in appreciable amounts for about the same period with all the preparations. These results are summarized in Table I, which gives the average of a number of observations in each case :—

TABLE I.

Preparations.	Dose in grammes.									
	0·3		0·6		1·0		1·5		2·0	
	Highest blood con. in mg. %.	Number of hours con. of 1 mg. % or over maintained.	Highest blood con. in mg. %.	Number of hours con. of 1 mg. % or over maintained.	Highest blood con. in mg. %.	Number of hours con. of 1 mg. % or over maintained.	Highest blood con. in mg. %.	Number of hours con. of 1 mg. % or over maintained.	Highest blood con in mg. %.	Number of hours con. of 1 mg. % or over maintained.
Aqueous solution ..	2·8	12	4·4	24	6·5	36	7·5	40	9·2	48
Hydnocarpus emulsion ..	2·4	12	4·1	24	5·1	36	6·7	40	9·1	48
Hydnocarpus suspension ..	2·5	8	4·0	24	4·9	36	5·3	40	7·2	48
Arachis suspension ..	2·5	8	3·8	24	4·9	36	5·4	40	7·8	48

(Results with suspensions containing wax have not been included, but results are similar to suspensions without wax).

It would, therefore, appear that there is some delay in absorption from the oily suspensions as compared to the watery solution or the emulsion. This gives rise to higher initial blood levels in case of the watery solution and the emulsion, but the delayed absorption from the oily suspension is not marked enough to enable the blood concentration to be maintained for longer than with the watery preparation.

This question has been studied in greater detail with a dose of 2 g. given in the various preparations with the same results. The average blood levels at varying intervals are shown in Table II, the average figure has been obtained from a large number of observations in each case :—

TABLE II.

Preparations.	Average blood concentration in mg. per cent at hours.							
	1	2	3	4	12	24	48	72
Aqueous solution ..	9·1	9·2	8·3	6·1	4·6	2·7	1·7	0·5
Hydnocarpus emulsion ..	9·0	9·1	8·7	6·6	4·8	3·4	1·2	0·5
Hydnocarpus suspension ..	6·0	7·2	6·5	4·5	3·3	2·1	1·2	0·0
Hydnocarpus suspension with wax	7·5	8·0	7·3	5·0	3·8	3·0	1·4	0·5
Arachis oil suspension ..	6·7	7·8	6·6	5·1	4·1	2·5	1·4	0·2
Arachis oil suspension with wax	7·1	7·4	6·9	4·8	4·2	2·7	1·3	0·5

It would be seen that with the watery solution and hydnocarpus emulsion, the average blood levels are definitely higher than those with the oily suspensions up to 8 hours ; at 12, 24 and 48 hours, also there is some difference in most cases. However, by 72 hours, all the preparations are at about the same level and nearing the end-point. It can, therefore, be concluded that the oily suspensions delay to some extent the absorption of sulphetrone, but this effect is not marked enough to enable blood concentration of the drug to be maintained longer than in case of the watery preparations.

Comparative blood concentrations after the oral route.—The comparison has been made both with single daily dose of 2 g. given by mouth, and three doses a day of 1·5 g., 1·0 g. and 0·5 g. given at 8-hour intervals.

The single daily dose of 2 g. by mouth produces an average concentration of 3 mg. per cent 4 hours after administration, it falls to 2 mg. per cent at 8 hours, to below 2 mg. per cent at 12 hours, and to 0·5 mg. per cent at 24 hours. The concentration curves after 0·3 g. and 0·6 g. given intramuscularly in aqueous solution show that 0·3 g. intramuscularly compares favourably with 2 g. given by mouth as regards the highest concentration reached ; and that 0·6 g. intramuscularly is definitely superior both as regards highest concentration and maintenance.

A comparison was then made with repeated oral doses of 0·5 g., 1 g. and 1·5 g. given three times a day, every 8 hours. With 0·5 g. t.d.s. the blood concentration remains about 1 mg. per cent with 1 g. t.d.s. between 1·5 mg. and 2 mg. per cent; and with 1·5 g. t.d.s. between 2·5 mg. and 3 mg. per cent. A single dose of 1 g. given intramuscularly as an aqueous solution is definitely superior to 1·5 g. three times a day by mouth (a total of 4·5 g.) ; a dose of 0·6 g. given intramuscularly is far superior to 1 g. given t.d.s. by mouth (a total of 3 g. per day). It would thus appear that to maintain the same blood concentration the dose required by intramuscular injection is about one-fifth of that required by oral administration.

EXCRETION IN URINE.

Appearance in the urine.—With all the doses and all the preparations sulphetrone appeared in urine in fair amounts in the first hour specimen (earlier specimens were not examined). With doses of 0·3 g. and 0·6 g. the concentration of sulphetrone in urine at one hour was usually between 15 mg. and 30 mg. per cent; with the higher doses it was usually between 50 mg. and 100 mg. per cent.

Highest concentration.—The excretion in urine continues to rise, and is at its height at 2 to 3 hours after the injection which corresponds to the peak hour in the blood concentration. The concentration in urine, however, shows marked variations since it depends on several factors, other than the blood concentration, such as amount of urine passed, the fluid intake and the extent of sweating. The highest concentration on smaller doses (0·3 g. and 0·6 g.) is below 100 mg. per cent, on bigger doses it is usually above 100 mg. per cent but varies from 50 mg. to 300 mg. per cent.

Maintenance of the urinary concentration.—After three hours the concentration of the drug in urine begins to show a gradual decline, and the end-point till which the drug can be detected in urine, varies with the dose. On an average a concentration of 2 to 5 mg. per cent is found at the end of 24 hours with a dose of 0·3 g., at the end of 48 hours with a higher dose up to 1 g. and at the end of 72 hours with a still higher dose up to 2 g.

The total excretion in urine.—It was investigated as to what percentage of the drug given intramuscularly could be accounted for in the urine. This investigation was done with the higher dose only. Two g. sulphetrone (in aqueous solution, hydnocarpus suspension and hydnocarpus emulsion) were injected into a number of patients and the total volume of urine passed by these patients in the successive 24-hour periods was collected till the end of six days, when the urine was found to contain little or no drug. Concentration of the drug in these samples was estimated and from it was calculated the amount of drug excreted every day from the kidneys. In case of the aqueous solution and hydnocarpus emulsion, 70 to 80 per cent of the drug could be accounted for in the urine in six days after intramuscular injection ; in case of hydnocarpus oil suspension, the total accounted for was slightly less. About half the drug is excreted in the first 24 hours, and the quantity passed during the successive 24-hour periods gets less and less. The marked variations in the drug concentration in urine which have been referred to earlier are seen mostly in the first 24 hours, as the excretion in the urine decreases, these variations get less and less marked.

THE RELATION BETWEEN THE BLOOD AND URINARY CONCENTRATIONS.

Although the urinary concentration shows marked variations, especially in the first 24 hours, the following conclusions can be made regarding the relation between the blood and urinary concentrations ;—

(i) The urinary concentration is at every stage much higher (on the average about 10 times higher) than the blood concentration.

(ii) The drug can be found longer in the urine than in the blood.

(iii) In general the relation between the blood and the urinary concentration is the same, whether the drug is given in watery solution, oily suspension, or oily emulsion. However, in case of the oily suspension the figures of excretion for 8 and 12 hours are closer together than in the case of watery solution and emulsion, where the 12-hour figures are considerably lower than the 8-hour figures. This would indicate a slight lag in the absorption of the drug from the oily suspension ; the lower blood concentration after injection of oily suspension also points to the same direction.

Concentration in skin.—It has been suggested by some workers that the concentration of sulphetrone in skin is much higher than in blood. This question has been investigated at different intervals after the injection, 2 hours, 3 hours, 8 hours and 24 hours after injection of 2 g. of sulphetrone in different vehicles. It has been found that at all stages and with the different blood levels, the blood and skin concentrations of the drug are almost parallel. The higher concentrations reported in skin by other workers could possibly be attributed to the use of novocaine or procaine as a local anæsthetic for taking the biopsy material. The use of cocaine for this purpose is free from this defect, and this was the anæsthetic used in the studies reported herein.

The concentration of the drug in the sweat was also investigated and was found to vary considerably with variations in the amounts of sweat secreted. It was usually between 0·1 mg. to 2·0 mg. per cent, but in highly concentrated sweat, it was sometimes much higher, up to 5 mg. per cent.

SELECTION OF THE PREPARATION.

As already stated, investigations were started with six preparations with a view to selecting the most suitable preparation or preparations. For the purpose of this selection the following points were to be considered:

(*i*) The height of the blood concentration produced after a particular dose given in various vehicles.

(*ii*) The maintenance of blood level and the longest period for which the drug can be detected in the blood.

(*iii*) Ease of injection.

(*iv*) Local irritation, induration, etc., at the point of injection.

(*v*) The effect on blood and the degree of anæmia produced.

In addition to these points there was another consideration, i.e. to find out whether combined treatment with hydnocarpus oil and sulphetrone is better than treatment with sulphetrone alone. A reply to this question, however, needs a long-term study.

No difference has been observed in their effect on production of anæmia by the different preparations. As judged by the other four criteria, the watery solution and the emulsion in hydnocarpus oil appear to be the best preparations. Both these preparations are easily injected and produce about equal blood concentrations which are maintained for about the same period. The choice between the two would depend upon the fact whether or not combined treatment with hydnocarpus oil and sulphetrone has any advantages over sulphetrone alone. If there was no advantage of combined treatment, the watery solution will naturally be preferred; if, however, the combined treatment offers any advantage, the choice would be the emulsion in hydnocarpus oil.

DOSE AND INTERVAL.

A discussion of the question of a suitable dose and interval for intramuscular injection will naturally involve a discussion of several questions such as: (*i*) a comparison of the concentration produced by the oral administration which is the common route so far for sulphetrone administration, (*ii*) the optimum blood concentration in the treatment of leprosy, (*iii*) the blood concentration produced and its maintenance by different doses of the drug given intramuscularly, and (*iv*) the time taken by the complete or almost complete elimination of the previous dose given intramuscularly.

There is no information on the optimum blood concentration; information on the other points has been summarized earlier in this report. On basis of these considerations, it would appear that perhaps the most suitable dose and interval is 1 g. given every day. On one hand, on considerations of poor blood condition of the patient, the daily dose may have to be decreased, to 0·6 g. or even 0·3 g.; on the other hand, if daily injections are not feasible, a higher dose may be given less frequently, e.g. 1·5 g. every other day or 2 g. twice a week. Groups of cases are now under treatment with the suggested doses in order to correlate the clinical and bacteriological improvement with the different doses suggested above. The groups are as follows :—

(*i*) 0·3 g. daily for 6 days (1·8 g. a week).
(*ii*) 0·6 g. daily for 6 days a week (3·6 g. a week).
(*iii*) 1·0 g. daily for 6 days (6 g. a week.)
(*iv*) 1·5 g. on alternate days (4·5 g. a week).
(*v*) 2·0 g. twice a week (4·0 g. a week).

CLINICAL IMPROVEMENT.

It is too early to report on the clinical improvement after intramuscular injections in comparison with the oral administration since the longest period of treatment has been only five months. The following general remarks on the clinical improvement are made:—

(*i*) After a short period of treatment of about a fortnight, the patients report that they feel lighter and look cheerful. In the patients who had ulcers this feeling may have been caused by the healing of the ulcers, but even the patients who had no ulcers reported this feeling of lightness ; this is possibly caused by some subsidence in the leprous infiltration.

(*ii*) Persistent temperature due to septic ulcers responds rapidly to treatment. The reactions in leprosy are gradually controlled; at first their frequency, severity and duration is decreased, the patient may get reactions unaccompanied by rise of temperature, and finally the reactions are almost controlled.

(*iii*) The leprous ulcers heal rapidly and it appears that the progress was more rapid than with oral administration.

(*iv*) Nodules and thick infiltrated areas of the skin begin to shrink after about a month's treatment. The rate of subsidence is slow but appreciable. In the limited period of treatment, none of the cases have so far become nodule-free. In a few cases, during the treatment fresh crops of small nodules appeared but subsided in a few days. In other cases nodules burst to form ulcers which healed very quickly.

(*v*) Leprous eye conditions and eye reactions respond very favourably to treatment. Before the treatment was started more than one-third of the cases had some sort of eye trouble which was causing them distress at varying intervals. After three months' treatment most of the cases with mild symptoms showed considerable improvement, there being no or slight trouble left. In the severer cases treatment for this period has been effective in reducing the frequency of pain and duration of eye reaction. One of the patients reported that while the reaction had been controlled the vision had become slightly more dim.

TOXIC EFFECTS.

There have been no marked effects during the period of treatment. In some of the cases the first few injections were followed by a rise in temperature to 100°F. or more. However, as the treatment was continued this rise of temperature after the injection disappeared. Nausea, weakness and giddiness were reported by a small number of patients during the early part of the treatment, but later these symptoms disappeared. Cyanosis was noted in one patient, a child of 11 years who was getting 2 g. sulphetrone twice a week ; it subsided after a few days withdrawal of the drug. He was later put on 1·5 g. bi-weekly and the cyanosis has not recurred. Exfoliative dermatitis was seen in two cases necessitating the temporary withdrawal of the drug and the administration of calcium gluconate.

No sign of liver damate has so far developed in any of the cases as judged by the Vandenberg test, aldehyde test for urobilinogen and Schlerenger's test for urobilin in urine.

EFFECT ON BLOOD.

The patients are given iron and yeast as a routine, because it is known that the sulphone drugs produce anæmia. Even with this routine administration of iron and yeast there is a tendency for the lowering of the r.b.c. count and hæmoglobin values. There is also a tendency for the mean corpuscular volume (m.c.v.) to be increased. However, deterioration in blood picture has only been slight so far, and only in a few cases was serious enough to demand the temporary withdrawal of the drug. This had to be done in cases where the initial blood picture was poor. It has been observed that small daily doses are well tolerated even by patients with a low blood level, with about 50 per cent r.b.c. and hæmoglobin.

BACTERIOLOGICAL IMPROVEMENT.

Repeated bacteriological examination of multiple smears in each case is being done with the object of following the bacteriological improvement and to find out whether the expression of the bacteriological results in the form of an index has any advantages over the usual method of expressing the degree of positivity by some such words as 'slight', 'moderate' or 'high'.

So far none of the cases have become bacteriologically negative, though in several cases there has been no decrease in the number of bacilli, and the bacilli show definite morphological changes. They become less acid-fast, several break down or become granular. It appears that the drug has definite action on the leprosy bacilli, although it takes a long time to eliminate the bacilli from the body.

CONCLUSIONS.

The quantity of sulphetrone needed by the intramuscular route would be about one-fifth of the amount required for oral administration. The treatment by intramuscular injections is, therefore, very economical and possibly more effective. In institutions where the giving of injections does not entail any extra cost, the cost of treatment by intramuscular injections would be about one-fifth of that by the oral administration of the drug.

PARA-AMINO SALICYLIC ACID (PAS).

The para-amino salicylic acid used in the study was kindly supplied by Messrs. Ward Blenkinsop & Co., London. Because of the limited quantity of the drug available, it has been tried in only two cases. Both were advanced cases of the lepromatous type, one of which was subject to frequent febrile reactions lasting for several days at a time.

A start was made with a dose of 20 g. daily of the para-amino salicylic acid as recommended for use in tuberculosis. The total daily quantity was given in divided doses every three hours during the day. On this dose both the patients complained of looseness of bowels, loss of appetite and a feeling of giddiness. The dose was, therefore, reduced to 15 g. a day and this has been well tolerated.

Both the patients have been under treatment for about six months. There has been some clinical improvement, but it appears to be less marked than with the sulphone drugs. The patient who used to suffer with frequent febrile reactions still suffers from reactions although not so frequently, and the reactions are now more or less afebrile. The other patient who had thick, red, localized lesions and was not subject to reaction has also improved inasmuch as the thickening and redness of the lesions have been considerably reduced. In neither of the cases has there been any decrease in the number of bacilli.

As judged by the results in these two cases, the para-amino salicylic acid appears to be less effective in the treatment of leprosy than the sulphone drugs. One advantage with treatment of PAS is, however, that it does not produce any anæmia. It is possible that combined treatment with sulphones and PAS may give better results. In the case of combined treatment smaller doses of sulphones would perhaps be needed and as such the tendency of the sulphone drugs to produce anæmia would be minimized.

2. Inquiry on the histopathology of leprosy under Dr. R. G. Cochrane at the Government Lady Willingdon Leprosy Sanatorium, Chingleput.

1. During the year, work on histopathological changes in leprosy has continued in collaboration with Dr. V. R. Khanolkar. As reported last year, there is evidence that the bacilli enter the skin and may lie latent in the corium. The first definite evidence of invasion is seen in the appearance of bacilli in the nerves. Further observations of interest have been made in this connection. Work by Figueredo and Desai, in collaboration with Dr. V. R. Khanolkar, on contacts of leprosy patients has revealed, by a special technique, bacilli in the skin. It is, therefore, suspected that the organism gains entrance into the corium and from there passes up the nerves. These studies are continuing.

The findings of our histopathological studies are throwing new light on the understanding of the disease. Leprosy, from the histopathological point of view appears to fall into two main tissue-reactional types—the *leproma* and the *lepride*. Maculo-anæsthetic leprosy may show essentially *leproma* histology in some instances, whereas in other instances the histology may be essentialy that of a *lepride*. By *leproma* histology is meant nerve infection with bacilli in the nerve, and the cellular reaction usually of the mononuclear type around the nerve; while the histology of the *leprides* shows an intense tissue reaction within the nerve. The term coined by Dr. Khanolkar last year, viz. dimorphous, fits in many important details, clinically and histologically, the atypical group of lesions. The basis of any classification of leprosy, therefore, must take these facts into account.

2. The report this year includes an account of our studies in sulphone therapy. This work has been greatly handicapped by lack of a trained biochemist. Experiments during the year have been continued with the following drugs :—

(*i*) The parent substance as a 25 per cent suspension in arachis oil.
(*ii*) An emulsion of sulphetrone (25 per cent) in groundnut oil.
(*iii*) An emulsion of sulphetrone (25 per cent) in hydnocarpus oil.
(*iv*) An aqueous solution of sulphetrone (25 per cent). (A 50 per cent solution of aqueous sulphetrone is also being used but this group is not included in these remarks as the period has not been of sufficient length to warrant this).
(*v*) Diasone by mouth.
(*vi*) Sulphetrone by mouth.

A detailed report on this work is in preparation, but the following are some of the points of major interest :—

(*a*) All the sulphones have some effect on leprosy. Improvement is most marked clinically. The bacteriological results in a significant proportion is less dramatic and frequently disappointing.

(*b*) If the following three criteria are taken in assessing the results of sulphone therapy in leprosy, i.e. (*i*) lepra-reaction, (*ii*) deterioration in clinical condition, (*iii*) deterioration of the bacteriologic index, the majority of cases pass through a phase of deterioration before improvement sets in. The value of the bacteriologic index as a method of assessment has been increasingly apparent during this year.

(*c*) There appears to be little relationship between stage of the disease and the rapidity of improvement as judged by the bacteriologic index. It may be that *M. lepræ* is more vulnerable in certain stages of its life-history than in others.

(*d*) There is no general uniformity of results. One patient may be clinically and bacteriologically more advanced and may show much more marked improvement than one who is relatively early lepromatous case.

(*e*) The advocates of smaller dosages of the sulphone remedies may be correct for, particularly in the case of di-amino-diphenyl-sulphone, dosages greater than 1·5 g. per week have a greater tendency to precipitate reaction. It may be that the effective therapeutic doses of di-amino-diphenyl-sulphone will be found to be as low as 0·5 g. to 1·0 g. per week.

(*f*) There is no evidence as yet that those passing through a reaction phase improve quicker than those who have never suffered from reaction.

(*g*) While the initial results showed a favourable bias towards di-amino-diphenyl-sulphone as the most effective remedy, more recent assessments indicate that sulphetrone in aqueous solution is as effective with the additional advantage that it is non-toxic and the least reaction-producing. This may be due to the fact that the total dosage is comparatively small (3·5 g. to 5·0 g. per week) and, therefore, only approximately one quarter (0·9 g. to 1·25 g.) would be available as di-amino-diphenyl-sulphone. This is assuming, as many contend, that sulphetrone to be effective must be degraded to the parent substance. It may, therefore, well be that the recent contention of some workers that the administration of the parent substance by mouth in small dosage is as effective and as little reaction-producing as aqueous sulphetrone by injection, may be correct. In this connection, however, the narrow margin of toxicity of di-amino-diphenyl-sulphone may constitute a serious drawback to its use as a routine remedy in leprosy.

(*h*) There is increasing evidence that sulphones are mainly suppressive and that, while a greater number of negative cases can be expected in the second and third year, there are a fair proportion of cases in which the bacilli persist in relatively small numbers as beaded forms, as acid-fast granules or even as acid-fast dust. If these granular forms are the only stages in the life-history of the *M. lepræ* and are present particularly when the environmental conditions are unfavourable, there is always a possibility that if the drug is stopped or the bacilli become resistent they may multiply months, and possibly years, later and the patient not respond to further sulphone treatment. Widespread indiscriminate sulphone therapy may be a danger which may not be apparent in the first few years.

(*i*) There is no evidence that sulphones combined with hydnocarpus oil are more effective than sulphones alone. In the case of the parent substance (di-amino-diphenyl-sulphone) suspension in hydnocarpus oil is apparently the most reaction-producing of all the sulphone preparations.

(*j*) While it is true that the reaction phase ultimately subsides unless there is a commensurate clinical improvement, our original suggestion that reactions may be beneficial possibly needs modifying.

While biochemical work has been continued, our detailed results await confirmation on the appointment of a Biochemist to the Unit. Nevertheless, there is evidence that the parent substance, di-amino-diphenyl-sulphone, may be retained in the tissue months after discontinuance of the injections. In two cases appreciable blood levels could be detected after 13 months and similar observations were made in a number of other cases after six months. The length of time of retention in the tissue is much less with emulsions of sulphetrone and still less with the aqueous solutions.

List of papers published on leprosy research.

DHARMENDRA. Some observations on sulphetrone treatment of leprosy (All-India Leprosy Workers' Conference, Calcutta, 1948).

DHARMENDRA. A note on the histopathology of leprosy (All-India Leprosy Workers' Conference, Calcutta, 1948).

DHARMENDRA. Some comments on the classification of leprosy recommended by the Havana Congress (All-India Leprosy Workers' Conference, Calcutta, 1948).

DHARMENDRA. Leprosy cases seen in the Indian Army ('Official History of the World War II', 1949).

DHARMENDRA. Diet and susceptibility to leprosy (National Nutritional Association of India, Calcutta, 1949).

REPORT OF THE LEPROSY ADVISORY COMMITTEE.

Held in the Central Secretariat, North Block, New Delhi, at 10-00 a.m. on the 23rd November, 1949.

MEMBERS.

Dr. C. G. Pandit.	(*Chairman*).
Dr. S. N. Chatterji.	
Dr. R. G. Cochrane.	
Dr. N. Figuerado.	
Dr. V. R. Khanolkar.	
Dr. Dharmendra.	(*Secretary*).

Lieut.-Colonel B. N. Hajra was unable to be present.

VISITORS.

Dr. J. C. Gupta.
Dr. K. V. Venkatraman.

I. (*i*) LEPROSY INQUIRY UNDER DR. DHARMENDRA AT THE SCHOOL OF TROPICAL MEDICINE, CALCUTTA.

(*ii*) INQUIRY ON THE HISTOPATHOLOGY OF LEPROSY UNDER DR. R. G. COCHRANE AT THE GOVERNMENT LADY WILLINGDON LEPROSY SANATORIUM, CHINGLEPUT.

Dr. Dharmendra and Dr. Cochrane presented the reports of the work done during the year 1949-50 on the respective inquiries under them. The work was mostly concerned with the parenteral administration of the sulphone drugs dissolved in different vehicles, with the object of evolving the most economical and the most efficient method of sulphone therapy specially suitable for conditions in India. The Committee felt that the results of the work have been very valuable.

Dr. V. R. Khanolkar gave an account of the histopathological work on leprosy carried out at the Tata Memorial Research Laboratories, Bombay.

The Committee expressed appreciation of the work done and recommended the continuance of both the inquiries for the next year, and requested Dr. Khanolkar to continue his valuable work on the histopathology of the disease.

II. CONSIDERATION OF THE QUESTION REGARDING THE PRODUCTION OF DIASONE IN INDIA.

The Committee considered the question regarding the production of sulphone drugs in India. In introducing the subject the Chairman gave a résumé of his memorandum on the need of re-orientating the policy regarding control of leprosy in the light of the results obtained with the treatment of the sulphone drugs. After a discussion on the various aspects of the question the following resolutions were adopted :—

(*i*) " It is recommended that the note prepared by the Chairman on the 'Treatment of leprosy patients in their homes as an approach to the control of leprosy in endemic areas' be suitably modified and submitted to Provincial Governments for their consideration in relation to a policy of establishing new treatment units in such areas. The Committee, therefore, feels that in view of the promising results of sulphone therapy, this treatment should also be made available to patients in their homes. The scheme as suggested in the note should be worked, in the first instance, in association with existing and well established leprosy clinics and sanatoria".

(*ii*) "For the purpose of securing at a reasonable cost sufficient supplies of sulphones, adequate to influence the prevalence of leprosy, the Committee is of the opinion that it is essential that steps should be taken for the manufacture, in the first instance, of di-amino-diphenyl-sulphone. A plant equipped for the manufacture of the drug should be capable of being easily adapted for the manufacture of other sulphone derivatives. The Committee, therefore, strongly recommends the establishment of a small scale plant for the purpose".

III. CONSIDERATION OF THE QUESTION OF SUBMITTING APPLICATION FOR A GRANT-IN-AID TO THE SURGEON-GENERAL, UNITED STATES PUBLIC HEALTH DEPARTMENT TO CARRY OUT LEPROSY RESEARCH IN INDIA.

The Committee read with interest a letter from the Medical Director of the Leonard Wood Memorial Foundation to Dr. Dharmendra. They were of the opinion that I. R. F. A. workers may be permitted to apply for grants-in-aid from recognized organizations so long as the applications were forwarded through the Secretary, Indian Research Fund Association.

IV. CONSIDERATION OF THE COMMENTS BY DR. ERNEST MUIR ON THE REPORT OF THE MEETING OF THE I. F. R. A. LEPROSY ADVISORY COMMITTEE HELD IN DECEMBER, 1947.

The comments by Dr. Ernest Muir on the report of the meeting of the Leprosy Advisory Committee held in December 1947 were recorded.

APPENDIX.

Treatment of Leprosy patients in their own homes—An approach to the problem of control of Leprosy in endemic areas.

By
C. G. PANDIT,
Chairman, Leprosy Advisory Committee & Secretary, Indian Research Fund Association.

1. The isolation of cases of leprosy as an effective means in limiting the spread of the disease had been recognised throughout the ages. In practice, however, insurmountable difficulties have been encountered in instituting this measure. The Health Survey and Development Committee drew pointed attention to these and noted as follows :—

> " The special Committee has pointed out that, in the past, the isolation of leprosy patients in their own homes was widely practised by the people in India but that the tradition has gradually weakened in the country as a whole and that it survives, as an effective measure only in one or two isolated parts of India, an example being the Kangra valley in the Punjab. Although attempts have been made to revive the practice, the Committee considers that the results have been disappointing and that 'from the experience so far gained, it cannot be said that home isolation holds out much prospect of being widely and properly practised.' "—(Vol. II, para 24, page 194.)

2. With the development of "leper asylums " or " leper settlements " in India chiefly through missionary efforts, institutional isolation of leprosy patients came very much into prominence. These asylums were intended initially for providing shelter to the most indigent of leprosy patients and this ideology to a great measure still persists. Once admitted, the patients remain there for the rest of their lives mostly due to the unsatisfactory nature of the treatment available. The asylums became permanent leprosy settlements, the inmates leading isolated community life of their own and engaging themselves in agricultural or weaving pursuits according to the facilities available. These efforts, however laudable, tended to create in the minds of the public an unfortunate impression. These institutions, therefore, became recognised as places for incarceration of leprosy patients and not as centres for treatment of the disease. The long and enforced sojourn in the asylum resulted in the complete uprooting of the patients from their homes and surroundings. Their absence was seldom felt and their return seldom awaited. The patients, so far as their families were concerned, were as good as dead.

3. After the re-introduction of the hydnocarpus (Chaulmoogra) oil derivatives fresh hope arose in the minds of the leprosy patients and during the years between 1925 and 1945 in the better equipped and staffed leprosy institutions a certain proportion of cases—varying from 40 per cent of the early cases to below 10 per cent when the cases were advanced—were discharged each year. This gave rise in the provinces, with a more enlightened leprosy policy, to an increasing expectation on the part of the public that the leprosy patient has a chance of returning to normal society. However, the turnover of patients

was to some extent limited and their rehabilitation a problem and, particularly if the patients were scarred, a real difficulty. Further the high relapse rate was most discouraging.

4. It is admitted that "isolation of the infective patients is the one measure which is advocated by all leprosy workers throughout the world as the most effective method for controlling the spread of the disease." This has been the guiding principle in the anti-leprosy programme of many countries in Europe and the Americas. It is understandable that this should be so for, in comparison with India, the number of cases needing isolation in those countries is small and the costs involved were within the means of their respective governments. In India the incidence of leprosy is of a different magnitude. There are over a million leprosy cases in the country which by some authorities is considered a conservative estimate. The disease has not shown a tendency to decrease in many areas and the results of surveys in South India have demonstrated that it is on the increase in some of the areas investigated. It is but natural that this should be so in view of a very small number of leprosy cases that have been isolated in the country in special institutions—there are only 14,000 beds available for the purpose according to the estimates of the Health Survey and Development Committee—and in view of the unhygienic conditions of living resulting in gross overcrowding of large groups of population.

5. Even so the Health Survey and Development Committee had advocated group isolation of cases of leprosy in certain selected areas, *in two specialized institutions to be established for the purpose, one for the isolation of infective cases and the other for the treatment of non-infective ones.* It was conceded that the period of isolation would be long, perhaps extending to many years, a factor which would also operate in the case of the limited night segregation advocated by Dr. Cochrane. The Health Survey and Development Committee did recognize that "the scheme if it is to be widely adopted should be sufficiently cheap to suit the economic level of the country and that provision will be required to promote corporate life in the isolated community and to enable the more able-bodied members of it to work and contribute towards the maintenance of the colony." It will seem, therefore, that the scheme as proposed by the Committee is only a modification of the existing ideology and is not in any way a departure from the accepted methods of control advocated for the disease. It is but natural that this should have been so in view of the knowledge then available.

6. In view of the discovery of the sulphone remedies, the time has now come for a re-orientation of policies in the matter of control. This is particularly the case as the sulphone remedies, in the advanced and moderately advanced infective cases of leprosy, have shown results far superior to the hydnocarpus remedies. With the use of these drugs it is now possible to contemplate rendering a large proportion of patients relatively innocuous as transmitters of the disease, within a two-year period. The clinical improvement in the majority is so marked that leprosy patients will be encouraged to persist with the treatment.

7. While the treatment with these newer drugs must be considered yet as being in the experimental stage the results obtained by several workers in India and abroad are sufficiently encouraging to suggest the use of these drugs in the prevention and control programme of the disease. This would be done in the first instance on an experimental basis. Researches in sulphone therapy have been conducted in two directions :—

(*a*) To ascertain the efficacy of the drug in the cure of leprosy.

(*b*) To investigate the most suitable as well as the most economic way of administering the drug.

8. It can now be accepted that in the majority of cases of lepromatous leprosy—especially in the more advanced forms which are the most infective—the institution of this therapy has resulted in effecting within a period of 9 months to 3 years such an improvement in the bacteriological state of the individual as to hold out the hope that he may not be infective. It is universally observed that the clinical improvement is greater than the bacteriologic, but even so a significantly greater number of cases become negative in a shorter period of time than with any other therapy. Whether the drug is curative or suppressive has yet to be ascertained and how long the patient should continue to receive this treatment after the negative bacteriological findings are established is yet to be investigated. It is admitted that not all patients receiving treatment will react so favourably, but it is expected that the majority of cases would, and so it is possible to utilise the findings recorded above in instituting a leprosy control experiment in selected endemic areas.

9. The drug is not without its toxic reactions. This danger is real when the drug is administered by mouth as the accumulation of the drug in the intestines and the possibility of its sudden absorption would raise the concentration of the drug in the blood stream to a toxic level. With parenteral administratiou of the drug particularly as an aqueous solution of sulphetrone, it is possible to avoid these conditions and to maintain adequate blood levels within safe limits, and diminish the chances of development of a toxic reaction considerably. It is not necessary here to give an indication of the dose to be recommended for the purpose but suffice it to say that these researches have opened up the field to a newer approach in the control of leprosy problem in India.

10. The following tentative scheme is suggested :—

(*i*) The scheme should be regarded as a research project in the first instance.

(*ii*) The scheme should be worked in association with an existing and well established leprosy clinic or a sanatorium in a suitable endemic area in the country.

(*iii*) A small number of villages not less than six situated in close proximity to the clinic or sanatorium should be chosen for the study.

(*iv*) It would be necessary to survey these villages in great detail to determine the exact incidence of leprosy. The contacts of infective cases should also be examined both clinically and bacteriologically.

(*v*) Half the number of villages should be chosen for sulphone therapy and the rest treated as controls.

(*vi*) Choice of the drug, the dosage and the mode of administration should be worked out in consultation with experts who have had some experience with this form of therapy.

(*vii*) Examination of patients at regular intervals should be conducted to assess the effect of treatment on the progress of the disease.

(*viii*) Provision should be made in the sanatorium to isolate for short periods such patients as show any toxic or unorthodox reactions. Facilities should be provided for routine biochemical investigations necessary in such cases. On subsidence of such reactions the patients should be discharged from the hospital and brought back to their own homes.

(*ix*) Periodic surveys should be undertaken to determine the effect of such measures on the incidence of the diseases in control and experimental villages.

11. The advantages of instituting such a control programme are obvious. The patients will be treated in their own homes and no elaborate measures for the isolation of cases would be necessary. If the treatment is begun with the most infectious cases in the first instance, the improvement that would be noticed in their appearance during the course of the treatment apart from any other clinical improvement, would be a sufficient incentive for other less advanced cases to volunteer for treatment. There will not be the same tendency for the concealment of the disease as heretofore. There will be no stigma attached to anyone who is to be admitted to the sanatorium. The problem of rehabilitation of these patients will not arise. Since the patients would get bacteriologically negative, it is expected that the spread of the disease would be arrested. It should be possible thus to achieve within a reasonable period of time what is expected to be achieved by other more orthodox conservative procedures previously suggested. If in addition to this a limited number of villages could be selected where sulphone therapy was applied, in addition to night segregation, effective comparison could be made between :—

(i) Village where sulphone therapy was used as the sole preventive measure.

(ii) Village where sulphone therapy was used as an additional preventive measure.

(iii) Village in which there are no preventive measures.

12. If this experiment proves to be successful the existing sanatoria would then be able to play their true role as leprosy hospitals much in the same manner as other hospitals for specialized treatment of such diseases, as tuberculosis, etc. The turnover of patients in them would be regular and continuous, obviating the necessity of establishing many such institutions in different areas in the country especially on the existing scale which includes the provision of ancillary facilities for agriculture, weaving, etc. And lastly the outlook of lay people towards the leprosy problem as a whole will change materially.

13. Only a limited staff will be required to initiate this experiment in selected areas, in the first instance. The only drawback to the scheme would be the provision of a sufficient quantity of the drug at economical prices. Dr. Cochrane has estimated the cost of the treatment of a case at somewhere about Rs. 35 per year, per patient. This is not exorbitant, and with the modified scheme suggested above, the cost may even be less. As regards the availability of the drug, it should not be difficult to make the necessary quantities of the drug available for at least initial observations and experiments. Later on the question of manufacturing such drugs in the country could be taken up. That it is possible to undertake the manufacture of this drug in the country, even at present has been indicated by Dr. G. Sankaran in his recent note on the subject.

14. It is admitted that the approach to the problem is not entirely scientific in that emphasis has not been laid on the permanent cure of the patients. Much progress has to be made yet in that direction. Nevertheless,

it is felt that such knowledge as we possess at present should not be withheld, but should be utilized in achieving our ultimate goal which is the prevention and eradication of leprosy. Indeed, the approach is not dissimilar to that advocated by the Indian Research Fund Association for the control of filariasis with Hetrazan.

15. It is quite likely that as research is advanced, many other drugs might be discovered for the treatment of leprosy. Already the Germans had under their consideration another drug Tibatin for the purpose. But if we are to wait for the introduction of a drug well tried and highly efficacious from the treatment point of view, we would have to wait long, and in the meanwhile leprosy would steal a march over our public health efforts, negligible as they are, at present. In any case the experience gained by instituting such an experiment now would prove of immense value in determining future policies when newer drugs are made available to us. It is necessary to bridge always the lag between knowledge and its practical application in the field.

PLAGUE.

1. Plague researches under the Director, Haffkine Institute, Bombay.

I. CHEMOTHERAPY OF PLAGUE.

(*a*) *Curative value of aureomycin in experimental plague infection in mice.*—Aureomycin is reported to exhibit a remarkable versatility as an antibiotic and exerts bactericidal activity against a wide spectrum of both Gram-negative and Gram-positive bacteria including penicillin-resistant, streptomycin-resistant and streptomycin-dependent organisms. It is also reported that the drug has the great advantage over the other antibiotics, as it can be administered orally and unlike penicillin and streptomycin the organisms do not develop any drug resistance with this antibiotic.

Experiments were, therefore, put up to assess its value in experimental plague infection in mice.

The requisite quantity of the drug suspended in 0·5 c.c. of 10 per cent gum acacia solution was given orally in different doses, 48 and 72 hours after infection. The drug was given every six hours but when the drug administration was started 72 hours after infection it was given every three hours during the first 12 hours only.

The results obtained are summarized in Tables I-*a* and I-*b* :—

TABLE I-*a*

Aureomycin.

Administration of the drug begun 48 hours after infection.

Experiment number.	Dose of the drug, mg.	Number of organisms in the test infective dose.	Number of mice used.	Deaths during the period of administration of the drug.	Deaths after the drug was stopped.	Percentage deaths after the drug was stopped.	Total number of deaths in percentage.	CONTROLS.	
								Mice used.	Deaths.
1	0·5 four times a day for 4 days	162	5	1(5·0)*	4(10·25)*	80	100	12	12(6·1)*
2	0·5 four times a day for 7 days	104	5	1(4·0)	1(24·0)	20	40	12	12(6·0)
3	1·0 four times a day for 7 days	160	10	1(5·0)	1(23·0)	10	20	10	10(5·7)

TABLE I–*b*

Aureomycin.

Administration of the drug begun 72 hours after infection.

Experiment number.	Dose of the drug mg.	Number of organisms in the test infective dose.	Number of mice used.	Deaths during the period of administration of the drug.	Deaths after the drug was stopped.	Percentage deaths after the drug was stopped.	Total number of deaths in percentage.	CONTROLS.	
								Mice used.	Deaths.
1	1·0 four times a day for 7 days	160	10	0	2(20·5)	20	20	10	10(5·7)
2	1·0 four times a day for 10 days	98	10	1(6·0)	0	0	10	10	10(5·5)
3	2·0 four times a day for 7 days	98	10	1(7·0)	0	0	10	10	10(5·5)
4	2·0 four times a day for 5 days	154	10	1(6·0)	1(20·0)	10	20	10	10(5·6)

*Figures in brackets denote the average number of days elapsing between infection and death.

(*b*) *Curative value of chloromycetin in experimental plague infection in mice.*— Chloromycetin, which is also reported to exhibit similar properties as those of aureomycin and which has become a drug of great promise in the treatment of typhoid fever, was also tried or its curative value in experimental plague infection in mice.

In this case, the drug was given orally, only 72 hours post-infection for a period of 7 to 10 days and the animals were observed for a total period of 31 days from the date of giving the test infective dose.

The results obtained are summarized in Table II:—

TABLE II

Chloromycetin.

Administration of the drug started 72 hours after infection.

Experiment number.	Dose of the drug mg.	Number of organisms in the test infective dose.	Number of mice used.	Deaths during the period of administration of the drug.	Deaths after the drug was stopped.	Percentage deaths after the drug was stopped.	Total number of deaths in percentage.	CONTROLS.	
								Mice used.	Deaths.
1	1·0 four times daily for 7 days	140	10	2(4·0)*	1(21·0)	10	30	5	5(5·4)*
2	1·0 four times daily for 10 days	104	10	1(5·0)	2(18·5)	20	30	10	10(5·6)
3	2·0 four times daily for 5 days	154	10	0	3(14·7)	30	30	10	10(6·1)
4	2·0 four times daily for 7 days	104	10	1(6·0)	3(21·0)	30	40	10	10(5·6)

*Figures in brackets denote the average number of days elapsing between infection and death.

In conclusion it may be stated that aureomycin is about as effective as streptomycin in experimental plague infection in mice and that chloromycetin seems to be less effective than either aureomycin or streptomycin.

II. STUDIES ON THE INHIBITION OF GROWTH OF THE PLAGUE BACILLUS.

Casein, hydrolysed with sulphuric acid and hydrochloric acid has been used as a medium for the growth of plague bacillus in this Institute. An interesting observation that has been made in this connection is that when such a hydrolysate was decolorized with decolorizing charcoal, the growth of the bacillus was found to be much superior in decolorized medium, i.e. 700 to 1,000 million organisms per c.c. as against 300 to 400 million organisms per c.c. in the undecolorized hydrolysate. Addition of charcoal adsorbate was found to inhibit the growth of bacillus to as much as 40 per cent. It is presumed that one specific amino-acid is responsible for this inhibition. However, it is proposed to chromographically separate the various fractions of the hydrolysate and to carry out a complete metabolic study of the nutritional requirements of the plague bacillus.

2. Plague inquiry in the Nilgiris district under the Director of Public Health, Madras.

The plague inquiry was continued in the Nilgiris district during the year along with the provincial scheme for the eradication of plague in the area. The object of the inquiry was to study the various factors connected with the endemicity of plague in the Nilgiris district and to evolve the most effective and economic methods of plague control.

INCIDENCE OF PLAGUE.

During the year 18 village units had rodent plague of which only 11 reported human cases (23 attacks and 8 deaths) as compared to 35 village units with rodent plague of which 16 reported human cases (33 attacks and 6 deaths) during the corresponding period of the previous year.

PLAGUE CONTROL.

(*i*) *Cyanogas fumigation.*—This has been the sheet-anchor in the control of plague. Rat-burrow fumigation in all the village units was carried out regularly once every three months irrespective of whether there was plague infection or not. Block fumigation of all houses was carried out in infected villages as soon as rat fall or human case occurred. Only in two villages fresh human cases occurred after the cyanogas fumigation and human cases did not occur in 7 out of the 18 infected villages.

During the period of one year from October 1948 to September 1949, plague infection occurred in 29 out of 468 village units which were regularly fumigated once in three months.

(*ii*) *Use of DDT in plague control work as compared to the use of cyanogas 'A' dust.*—

(*i*) (*a*) : The results of the experimental use of 10 per cent DDT powder in plague control work in a village have already been reported last year. Ten per cent DDT powder was insufflated into all the rat-holes and rat-harbourages of this village (Wellington) in the third week of June 1947 and since then left for observation without any other treatment. A control village (Aruvenkadu) was also selected having the same general flea indices before the DDT treatment. Monthly rat-flea surveys are being done in these two villages since July 1947 for a period of 28 months and the detailed results are shown in Table I. It will be noticed that, while the general flea indices of the control village is on an average of three and above, that of the DDT-treated village is below 1 and this reduction (below) is maintained for a continuous period of nearly 16 months, after only a single application of 10 per cent DDT dust. Thereafter, the monthly general flea index tends to rise and nearly after 28 months it is still below 2 generally and very much below the figures in the control village.

(*b*) : Coonoor town is being fumigated with cyanogas once every two months since September 1946. A monthly rat-flea survey is done in Coonoor from October 1948 and the general flea indices are given in Table I. It will be noticed that the monthly general flea indices are high in spite of repeated fumigation once every two months.

(*ii*) (*a*) : The experimental use of 10 per cent DDT dust was extended to 90 village units in Ketty valley which is perhaps the worst locality for plague infection in the Nilgiris district. The DDT treatment of these villages was carried out during a period of nearly one year from 16th February, 1948 to 25th January, 1949. It is nearly 21 months since DDT was used in 12 village units and nine months since 20 village units were similarly dusted with DDT. In the other 58 village units the corresponding periods have ranged from 19 to 13 months. In none of them plague infection has, however, occurred during this period although in previous years these village units were the worst plague infected localities.

In two of these villages (Ketty and Hullada) rat-flea surveys are being done and the monthly general flea indices show a marked reduction to below 1 for a period of one year and below 2 after that period. The results again confirm the previous observations.

(*b*) : While plague infection did not occur in any of the 90 village units treated with DDT only once, from March 1948 to the end of October 1949 ; plague infection occurred in 45 out of a total of 468 village units fumigated with cyanogas once every three months regularly during the same period.

(*iii*) (a) : To treat the above 90 village units once in one year it required 2,000 pounds of 10 per cent DDT dust, which at annas 14 a pound cost Rs. 1,750.

(*b*) : To fumigate the same 90 village units, once, it would require about 1,000 pounds of cyanogas dust. Since these villages are being fumigated once in 3 to 4 months it would require 1,000 × 3 = 3,000 pounds of cyanogas A dust during an year. The cost of cyanogas A dust being Rs. 1-8-0 per pound the total cost of the material alone will cost Rs. 4,500 as against Rs. 1,750 for DDT dust.

CONCLUSION.

1. It will be seen from the above observations that we have at present in DDT a pulicide which is extremely effective and whose residual action lasts for nearly 16 months after a single application and still keeps the general flea index below 1.

2. While a single application of 10 per cent DDT is generally sufficient in a place for about a year to keep the flea index below 1, three to four applications of cyanogas are necessary for the same place during the same period.

Consequently, the expenditure on account of cyanogas is about 2½ to 3 times that of DDT. There will be considerable saving in the expenditure if DDT is used in place of cyanogas apart from the more efficient results obtained by the use of the former.

3. While plague has occurred in 45 out of 468 village units regularly fumigated with cyanogas once every three to four months, all the 90 village units treated with DDT escaped infection during the same period.

(*iii*) *Use of gammexane in the plague control work.*—Gammexane has not been tried so far in the field in any place in India for plague control work. A preliminary experiment was, however, carried out in a small detached portion of a village.

At the request of the Imperial Chemical Industries, agents for gammexane, it was decided to try the effect of gammexane in the field. For a fair trial in a village, order was placed for the following formulations of gammexane :—

Gammexane smoke generators No. 2, 2 oz. pellets ..	200
" " " No. 12, 1 lb. canister ..	25
Gammexane dust D. 0·25	400 lb.
Gammexane water dispersible powder P. 520	100 lb.

The company could not send all the above quantity free of cost and an extremely small quantity of the product was sent for trial. The following formulations were received :—

Gammexane smoke generators No. 2, 2 oz. pellets	12
" " " No. 12, 1 lb. canister	1
Gammexane dust D. 0·25	20 lb.
Gammexane dust P. 520	10 lb.

This very small quantity of the gammexane formulations was insufficient for any sort of field experiments. As the required quantity of the product was not received, it was planned to carry out the experiment in an isolated part of a village called Mukkatty, while keeping the main village Elithorai and the adjacent village Edapally as controls.

Rat-flea surveys were carried out in these three village units from October 1948 and the general rat-flea indices are given in Table II.

Gammexane dust was insufflated into all the rat-burrows (160) and dusted in the rat-runs of all the 36 houses and eight cattle-sheds in Mukkatty which is an isolated part of a village between 19th January, 1949 to 20th January, 1949. The smoke pellets were used in houses which could be rendered air-tight as far as possible.

Rat-flea surveys in the treated village as well as the control villages have been continued since then. These village units under experiments had not been subjected to any other plague control measures like fumigation, etc., for about six months previously. They have also not been subsequently fumigated since then.

As compared to the control villages the general rat-flea index in the gammexane-treated part of the village is definitely lower as can be seen from Table II. But we do not see the marked reduction in the flea index as in the case of villages treated with DDT. The general flea indices of the gammexane-treated village as well as the control villages are given in Table II. No reliable conclusion can, however, be drawn from this very small field experiment as the quantity of the product experimented with, is only 30 pounds, the number of premises treated only 44 and the materials for rat-flea survey, such as rats and fleas obtained from the treated portion of the village are small and insufficient for experimental purposes. It is proposed to carry out field experiments in a fair-sized village as soon as sufficient quantity of gammexane is made available by the company.

SYLVATIC PLAGUE.

Wild rodents (*Gunonys kok*), numbering 264, were caught and examined for evidence of any plague infection in them. Post-mortem and microscopic examinations of their spleen smears were all negative. Their crushed spleen pulp was rubbed on to susceptible rats, but all of them were negative to plague. A total of 394 fleas was also collected from the bodies of these rodents and their distribution was as follws:—

X. astia	0
X. cheopis	5
X. brasiliensis	7
Leptosylla musculi	5
Pulex irritans	3
Ctenocephalus	1
Ceratophyllus nilgriensis	81
Stivalius	292
Total	394

The pooled emulsions of these fleas were injected into susceptible rats but all of them were negative to plague.

TABLE I.

The monthly general rat-flea indices of control village, DDT-dusted village and cyanogas-fumigated town.

Month and year.	Control village.	10 per cent DDT experimental villages.			Cyanogas fumigated town.	Remarks.
	Aruvenkadu.	Wellington.	Ketty.	Hallada.	Coonoor.	
January 1947 ..	..	2·7	..	..	..	10 per cent DDT powder was insufflated into rat-holes between 16-6-1947 and 21-6-1947.
February 1947 ..	..	3·2	..	..	..	
March 1947 ..	5·3	4·3	..	..	..	
April 1947 ..	3·6	3·1	..	..	..	
May 1947 ..	..	..	..	..	..	
June 1947 ..	..	..	..	..	..	
July 1947 ..	3.6	0·65	..	..	..	10 per cent DDT powder was insufflated into rat-holes between 16-2-1948 and 25-2-1948.
August 1947 ..	4	..	..	..	..	
September 1947	5·6	0·3		..	..	
October 1947 ..	6	0·1	..	..	..	
November 1947	6·6	0·1	..	..	..	
December 1947	7·8	0·4	..	..	..	
January 1948 ..	2·3	0·1	..	..	..	10 per cent DDT powder was insufflated into rat burrows between 17-3-1948 and 23-3-1948.
February 1948	2·2	..	3·8	..	..	
March 1948 ..	5·7	0·7	..	1·8	..	
April 1948 ..	..	0·2	0.3	..	..	
May 1948 ..	..	0·3	..	0·4	..	After repeated cyanogas fumigation once every two months.
June 1948 ..	..	0·3	..	..	..	
July 1948 ..	1·3	0·1	0.1	..	..	
August 1948 ..	3	0·6	0.2	..	..	
September 1948	2	0·5	..	0·1	..	
October 1948 ..	..	0·7	..	..	1·3	
November 1948	..	1	..	..	0·83	
December 1948	1·8	1	..	..	1·8	
January 1949 ..	5	1·2	..	..	4·5	
February 1949 ..	3·4	2·6	..	..	2·2	
March 1949 ..	3·4	1·7	..	..	3·4	
April 1949 ..	..	..	1·7	..	..	
May 1949 ..	3·4	1.1	..	0.7	4	
June 1949 ..	..	..	0·8	..	5·7	
July 1949 ..	5·2	3	..	..	6·6	
August 1949 ..	4·6	1·5	1	1·4	4·1	
September 1949	..	..	1·1	1·7	..	
October 1949 ..	2·4	1·6	..	..	3·4	

TABLE II.

Monthly rat-flea indices of gammexane-treated village compared to control villages.

Month and year.	Gammexane-treated village.	Control villages.	
	Mukkatty.	Edappally.	Elithorai.
	Before gammexane treatment		
October 1948 ..	1	0·5	0·9
November 1948 ..	..	0·5	0·9
December 1948 ..	3·7	1·8	1·9
January 1949 ..	Gammexane treatment on 19/20-1-1949	..	1·6
February 1949 ..	0·9	..	..
March 1949 ..	0·9	2·7	2
April 1949 ..	..	..	..
May 1949 ..	0·5	2·4	3
June 1949 ..	..	..	..
July 1949 ..	0·7	2·6	7·6
August 1949 ..	..	..	..
September 1949	0·55	2	1·6
October 1949 ..	..	..	..

3. Inquiry into the epidemiological factures of plague in Calcutta under Drs. R. B. Lal and S. C. Seal at the All-India Institute of Hygiene & Public Health, Calcutta.

OBJECTIVES.

The proposed investigation was aimed at the elucidation of the various problems connected with the five main factors, viz. the plague organism, the rat, the flea, the man and the environment and their interaction. Although certain facts about each party were known a lot had yet to be studied and many close and accurate observations made to note the cyclic changes in their mode of life and environment which generally bring about changes in the natural phases of the disease, i.e. epidemic, pandemic, endemic, interpandemic and interepidemic phases.

PROGRESS OF STUDIES.

1. 1948 *outbreak.*—The first case of plague diagnosed bacteriologically was on the 17th April, 1948 in ward 8, but the first clinical case was traced to have occurred in the same area in the first week of March 1948. The peak of the outbreak was in the last week of April which gradually tailed off by the middle of May and practically disappeared by the end of June. During the second half of the year only 15 cases were notified. There were altogether 276 suspected cases of plague ; 173 were clinically consi-

dered as plague for which bacteriological confirmation was obtained in at least 35. The remaining 103 were non-plague cases. There were 23 deaths, the fatality-rate being 13·3 per cent. There was no marked rat-fall. Of the 5,000 rats examined plague infection was detected in 19 instances. It was *R. rattus* in seven instances and *R. norvegicus* in the rest. The general flea index varied between 0·59 and 4·79, while the *cheopis* index was generally very low, i.e. below 0·5 per cent, the highest being 1·4 during the peak of the epidemic.

The severe cases mostly occurred at the initial stage. Subsequently, there was a much larger incidence of milder cases some of which remained clinically doubtful, while only a few were serious. The organisms in the spleen smears of rats as well as in the smears from human buboes were, in most instances scanty. Thus, in this outbreak there was mildness of disease interspersed with a few severe cases, a common feature noted in the early phase of the last Calcutta epidemic and their origin, distribution and seasonal incidence were very similar. Another point of importance was the wide distribution of the disease in different parts of the city within a very short period of time and the detection of plague infection in rats received from eight of the 32 wards. Also, neither the incidence of the black house rat nor the number of suitable variety of rat-fleas were found more favourable for the spread of plague now than during the non-epidemic period in 1936 when Dr. Raghavendra Rao studied the plague situation in Calcutta. Thus, taking everything into consideration it appeared that one of the most important factors in the pandemicity of plague was the dissociation of the plague organism which perhaps periodically threw out variants or aberrant forms (like locusts do) which were suited to pandemic extension and that migration of rats was involved in the local spread.

2. 1949 *outbreak.*—This year the cases began to appear towards the end of February, i.e. earlier than in 1948. Till the end of August 1949, 434 cases were noted of which 411 were admitted into the Campbell Hospital and 23 including 13 pneumonic cases were otherwise detected. The total number of non-plague cases was 123, the rest were considered clinically as plague. Altogether 77 deaths were recorded including 13 from pneumonic plague and three from non-plague. The average fatality rate being 23·8 per cent and excluding pneumonic cases 20·5 per cent, i.e. higher than that of last year. Suspected human cases were removed from practically every ward of the city except 17 and 30. Several new areas were also involved this year, e.g. Howrah, Hooghly, Baranagar, Dum Dum and Cossipore.

Bacteriological examination of gland smears or of gland and blood culture was done in 396 cases (i.e. 91·2 per cent of total cases). *P. pestis* was isolated and identified in 73 instances only. Sulpha drugs being administered in some cases before admission might be responsible for a few negative results. Organisms were actually seen in gland smears in a state of degeneration following streptomycin administration. Of the 74 deaths due to plague then the total number of confirmed cases of plague was 116 (73-31-74) i.e. 37·3 per cent of 311 cases clinically considered as plague. Although the animal inoculation test directly from gland-puncture material was not done in the majority of cases there were reasons to doubt the clinical diagnosis in certain number of cases.

The characteristic feature of this year's outbreak was an early appearance in February and late subsidence in July, the case being almost uniformly distributed during the months of March, April, May and June. The rat-fall in some areas was fairly heavy, e.g. in Howrah and ward No. 5. Nearly 125,000 rats were examined this year and only in 29 instances rats were found plague-infected, including at least eight instances of live rats. These figures were not, however, true indices of infection amongst rats as examination on a wide scale was not possible with the limited staff at our disposal and was carried out by pooling the spleens of rats caught in every cage. However, out of 19 rats examined individually, 9 were *R. rattus*, *R. norvegicus* and 5 *Gunomys*. The infected rats were distributed as follows in order of frequency : Calcutta ward Nos. 5, 8, Howrah, ward No. 1, Calcutta ward Nos. 23, 7, 19 and 9.

The general flea index was even lower than last year, the highest being 3, whereas the *cheopis* index was generally higher but rarely exceeding 1 per rat (highest 1·7) although the number of fleas in an individual rat was found as high as 70 during the peak of the epizoötic. During the period between the week endings 26th February, 1949 and 26th March, 1949, the *cheopis* indices were relatively higher than those of *astia*. This was significant though both the indices declined after that period.

Incidence of pneumonic plague in Calcutta.—Towards the end of March 1949 a young man belonging to Kidderpore area of Calcutta where bubonic plague was occurring travelled to his native village, Bankul, 14 miles away from Howrah, and developed symptoms of what was considered later as plague pneumonia. Thirteen other cases followed among the close contacts, i.e. 11 belonging to a family of 31 and 2 extra-familial (one compounder and one attending woman) ; 8 were males and 6 females. The last case in the series occurred on 6th April, 1949. Only one case, which was diagnosed in time in the hospital and treated with streptomycin, survived. *P. pestis* was isolated from two cases in the chain admitted into the Calcutta Medical College Hospital. All cases more or less showed signs of lung involvement with soft and mostly non-productive cough. The incubation period was short (2 days) and fatality rapid. A large number of contacts including attending physicians and nurses escaped infection. Besides these, two other cases turned into pneumonic type in the city but the timely intervention prevented the spread of the disease.

The combined experience of the two outbreaks in regard to certain general features—(corrected up to the 31st May, 1949).—Out of 414 clinical cases of plague only 11 or 2·7 per cent were residents for less than 13 days in the city. The percentage distribution of patients according to the province of origin was as follows : Bengal 34·5, Bihar 31·4, U.P. 9·4, Merwar 7·7, East Pakistan 6·7, Orissa 4·5 and the rest 5·7. Nearly three-fourths (74·7 per cent) of patients were Hindus, 23·7 per cent Muslims and only 1·3 per cent Christians. The majority of cases, 36·5 per cent, belonged to the 'at home' and 'at school' groups. Artisans and labourers constituted 27 per cent ; tradesmen, shopkeepers and vendors altogether formed 11 per cent ; clerks 2 per cent and miscellaneous 23·5 per cent. Only 11 per cent of the cases were below 10 years of age, 13 per cent between 10 and 25 years, 52·8 per cent between 26 and 30 years, 18·4 per cent between 31 and 45 years and only 4·6 per cent were above 45 years of age. Of the 362 families affected only 27 or nearly 7·5 per cent families had multiple cases, i.e. 18 families having two cases each, 3 families three cases each, 5 families four cases each and one family had 11 cases (pneumonic).

Economically as high as 91·4 per cent of the cases belonged either to the poor (65 per cent) of lower middle class (26·4 per cent).

Altogether 366 houses were involved, i.e. 310 residential houses, 12 trading shops, 16 godowns, 5 market-places, 2 grocer's shop, 2 mess or hostel and 19 others. Of these 45 per cent were masonry structure, 31·6 per cent had tiled roof, 16·3 per cent sheet-iron roof, 4·3 per cent thatched roof and 2·5 per cent were other types. Only 7·5 per cent were clean, 38·3 per cent fair and 54·2 per cent dirty. While only 46·4 per cent of clean houses were rat-infested, 74·7 per cent of the fair and 77 per cent of the dirty houses harboured rats. *R. rattus* was found in 82 per cent of the houses, sewer and field rats in 39·6 per cent and mice in 31·7 per cent of houses. Only 20 per cent of houses gave history of rat-falls. Amongst the patients 53·4 per cent were sleeping on the floor and 45·4 per cent on *machans* or cots and only 1·3 per cent on bedsteads ; 54·2 per cent had less than 18 sq. ft., 27·5 per cent less than 36 sq. ft., 13·6 per cent less than 50 sq. ft., and 4·3 per cent more than 50 sq. ft. of floor space per person.

Clinically, 138 cases or 36·3 per cent were mild, 109 or 28·7 per cent were moderately severe and 133 or 35 per cent severe ; fever was present in 89·4 per cent of cases, pain in 23·5 per cent, headache in 10 per cent ; delirium in 4 per cent and pneumonia in 4 per cent. Glands were enlarged in 94·6 per cent of cases (inguinal) (including femoral) 56·3 per cent : cervical 23·3 per cent ; axillary 17 per cent and other glands 13·5 per cent. Of the 414 cases 385 or 93 per cent was bubonic, 15 cases or 3·6 per cent each of septicæmic and pneumonic type. The total number of deaths was 80, the average fatality rate being 19·3 per cent (16·75 per cent excluding pneunomic plague). The fatality rates of the bubonic, septicæmic and pneumonic types were 14·8, 60·0 and 93·3 per cent, respectively. Cases were removed from all the wards of the city of Calcutta, except ward No. 16 and from Howrah, Hooghly, 24-Perganas, Baranagar and Alambazar, etc. The worst affected places were Howrah, Calcutta wards Nos. 5, 8, 9, 7, 28, 4, 11 and 19 in order of incidence.

Thus, compared to 1948 this year both human cases and rat infections were on the increase. Bacteriologically positive cases were found in larger number and the outbreak though not yet in the true epidemic phase was more sustained. Another new feature noted was the larger number of organisms in the spleen and gland smears ; in fact, blood smears also showed plague organisms indicating more septicæmic cases, which in at least 3 instances turned into plague pneumonia giving rise to 15 cases of pneumonic plague. In 8 instances infection was detected in live rats. These results have been obtained with the very limited staff and in spite of the active control operations. Thus, infection among rats was probably more extensive than the figures would suggest. From the observations made above although it would still be not without a hazard to make any forecast about the future happenings, this year's findings provide further evidence of the evolution of the disease towards an epidemic proportion. At any rate, a very close watch of various factors concerned must be continued for a considerable time.

SPECIAL STUDIES.

The organism.—About 80 strains of organisms have been collected in 1949 from human cases and rats at different stages of the outbreak and are being preserved in the cold in sealed rabbit-blood agar-tubes. Their morphological, biochemical and serological characters are under investigation. The mouse herd is not sufficiently large to undertake the virulence test. A preliminary test carried out last year with five human strains, one strain from a live rat and one from a dead rat, gave the following results:—

Human strains.—Ts 4·5, 19, 20 and 45 : These strains were isolated by the Department of Bacteriology, School of Tropical Medicine, Calcutta from patients admitted into the plague wards of the Campbell Hospital during the month of April 1948. Colonies on rabbit-blood agar were typical except in case of Ts 4 which yielded two kinds of colonies on first subculture, viz. (*i*) rough button-shaped with fringe and (*ii*) small round slightly rough, the proportion of colonies of (*i*) to (*ii*) being 1 : 9. The virulence of these organisms as tested by Sokhey's method was found different from one another, Ts 5 being most virulent and Ts 4 the least, as given in Table I.

Strain R. 2861.—This strain was isolated on 15th July, 1948 from a live rat caught in ward No. 4 as small sticky dew-drop-like colonies on blood-agar. It gave typical sugar reactions except xylose which was not fermented and doubtful slide agglutination with known anti-plague serum. 0.2 c.c. of 48-hour broth culture given intraperitonially failed to kill mice. From the subculture several colonies were picked and the virulence tested again ; colony No. 3 gave more or less typical character. One of its substrains 2861 (3c) killed mice with 6 million organisms in 4 days. *P. pestis* was recovered from the spleen but the culture from heart's blood was negative.

The spleen emulsion was injected into a second set of mice which died on the third day showed organism both in spleen and heart's blood, indicating an apparent increase in virulence by passage. On the other hand, the strain 2861 (2) showing small colonies failed to kill mouse with as high a dose as 40 million organisms.

Strain R. 3959.—This strain was isolated on 20th November, 1948 from dead rat of ward No. 7. It gave typical sugar reactions and was serologically positive. Approximately 600 organisms killed mice within 4 days, and the organism was recovered from both heart's blood and spleen culture.

The above results are summarized in Table I:—

TABLE I.

Characteristics of certain plague strains including their virulence.

Strains.	Isolated from.	Date of isolation.	Colonies on rabbit blood agar.	Sugar reaction.	Serological reaction.	Number of organisms.	
TS 4	Human bubo	April 1948	1. Rough button-like with fringes. 2. Small round, slightly rough.	Typical, delayed Typical	Granular Floccular	42	Method and m.l.d. calculated according to Muench's method.
TS 5	"	"	Typical, smooth.	Typical	Floccular	9	
T 19	"	"	Small colonies.	"	"	43	
TS 20	"	"	Typical.	"	"	12	
TS 45	"	"	Typical.	"	"	19	
R 286(1)	Live rat	July 1948	Rough, button-like with fringes.	Xylose not fermented	. .		Failed to kill mice with 25 million.
R 2861(2)	"	"	Small, rough.	Typical	Weak		Failed to kill mice with 40 million.
R 2861(3)	"	"	Medium-size colony.	Xylose not fermented	"	. .	
R 2861(3c)	"	"	Smooth type with central opacity.	Typical	Floccular	6 million	Doses lower than 6 million were not tried.
R 3959	Dead rat	November 1948	Moderate-size colony with fringes.	"	Weak	600	Doses lower than 600 organisms were not tried.

The above results suggest the existence of some aberrant, avirulent for dissociant forms under natural conditions, which may have an important bearing on the rise and fall of an epizootic. It may be mentioned here that Seal (1942-43), while working at the Haffkine Institute, Bombay with a variety of laboratory plague strains, anticipated the existence of such dissociant forms under natural conditions.

DIAGNOSIS OF PLAGUE.

In the earlier phases of the plague epidemic many cases were admitted into the hospital on suspicion mostly with mild symptoms. Again, a large number of cases was sent to the hospital after a partial treatment with sulpha drugs and even streptomycin. The confirmation of clinical diagnosis bacteriologically in such cases was unsuccessful ; and yet it was of utmost importance from the point of view of (*a*) extension of disease into a new area, (*b*) control measures, (*c*) research projects in relation to positive and negative plague cases, (*d*) trial of specific drugs and (*e*) case fatality rate and other statistical data particularly those related to international intelligence service. So an attempt was made to evolve a serological test to confirm the diagnosis.

(*i*) *Agglutination test.*—Sera from 140 suspected cases of plague were collected mostly during convalescence. The agglutination test with these sera, as evolved by Seal (1941-42), yielded very interesting results. Only 32 of the above sera gave positive agglutination test. This included all the bacteriologically positive cases from whom sera had been collected, six cases which were not bacteriologically confirmed and three cases which gave definite history of anti-plague inoculation but clinically doubtful of plague infection. It is, however, not yet known how early this antibody appears in patient's blood. This should be observed during the next opportunity.

(*ii*) *Complement-fixation test.*—A still more delicate and specific serological test is being developed for the early diagnosis of plague infection. Seal (1941-42) isolated specific soluble protein of plague bacillus at the Haffkine Institute, Bombay. The fraction isolated by one-third saturation with Na_2SO_4 was found to contain the specific substance exclusive for the protective plague organism. When this substance was used as an antigen it gave a very sensitive and highly specific complement-fixation test with anti-plague sera. This antigen has already given very encouraging results with the patient's sera. The standardization of the method is under progress.

The development of the above serological tests have actually opened up many new lines of studies. For instance, these could be utilized as tests for estimating the level of immunity both before and after anti-plague inoculation. A preliminary work in this line is under way.

MIGRATION OF RATS.

The importance of this investigation has already been emphasized. Since previous work in this respect was virtually absent it took some time to evolve a suitable method to carry out the experiment. In spite of a very small field staff at our disposal certain experiments have been carried out giving encouraging results. For, in three instances the tattooed rats have been recaptured, 6 to 8 miles away from the place of release. Interesting results are expected if the work is carried out on a more extensive scale.

STUDY OF THE IMMUNITY STATUS OF THE LOCAL RAT POPULATION.

The rooms required in the Control Unit Laboratory of the Government of West Bengal where large numbers of rats are brought from different sections of the city, have just been provided and the testing is in progress but perhaps the state of immunity of the local rat population at the beginning of the outbreak has already been missed due to the delay in obtaining the necessary facilities.

EXAMINATION OF RODENTS.

This has already been mentioned. Since it was not possible to collect a large number of dead rats, only a low estimate of the infection amongst them could be made. The percentage distribution of the different species based on 1948 figures were as follows :—

R. rattus 8·1 per cent, *R. norvegicus* 17·8 per cent, *Gunomys varius* 68·8 per cent, *Bandicoota Ind.* 4·5 per cent and musk rat 0·6 per cent. The corresponding figures given by Rao were 13·5, 22·0, 27·3, 0·7 and 5·5 per cent, respectively. Fortunately, black house rat is proportionately much lower here than in other cities in India. The rodent densities and distribution by species and wards have been worked out for 1948. It varied from the lowest 3·2, recorded in ward No. 15, to the highest 91·7 in ward No. 2. Densities above 50 were recorded in at least six of the 32 wards of the city. They were not, however, related to plague incidence. The density of *R. rattus* was below 1·0 in at least eight wards and above three in 9 wards, the maximum being 13·4 in ward No. 4. A more detailed study is likely to yield some interesting results.

STUDIES ABOUT FLEAS.

A regular examination of fleas for working out the indices by species have been carried out since the beginning of the outbreak in 1948. More than 15,000 fleas have so far been examined. On the whole, the general indices have been lower than what were found by Rao during the non-epidemic period in 1936, although during the main periods of epidemic rise, both in 1948 and 1949, *cheopis* index was relatively higher than that of *astia*. A difference was, however, noticed in the indices worked out by species of rats and by season as given in Table II :—

TABLE II.

Flea indices by species of rats and season (1948).

Species of rat.	April to May 1948 indices.			June to September 1948 indices.			Rao (1936) indices.		
	Cheopis	*Astia*	General flea.	*Cheopis*	*Astia*	General flea.	*Cheopis*	*Astia*	General flea.
R. rattus ..	4·91	4·84	9·75	0·68	0·78	1·46	3·0	1·3	4·3
R. norvegicus	2·63	2·96	5·59	0·68	2·08	2·76	3·3	6·1	9·4
Gunomys varius	0·23	0·99	1·22	0·08	1·71	1·79	1·4	3·7	5·1
Bandicoota Ind.	0·66	0·83	1·49	0·0	0·05	0·05	0·7	3·3	4·4

The records given in Table II show a rise of the flea indices in *R. rattus* and a corresponding fall in *R. norvegicus* during the epidemic phase in contrast with Rao's observation during the non-epidemic period in 1936. Also there was fall in these indices, particularly of *cheopis*, in both the species during the quiescent period of June to September 1948. An analysis of the data by species, season and wards would, therefore, be interesting.

Perisitence of infection in fleas.—Twenty burrows were dug up in June 1948 in the various wards of the city and 127 rats including 16 young ones were found in 11 of them. Sixty-two fleas were recovered only from three of the burrows, 53 of which were identified as *astia* and the rest were not identified. Cultural examination yielded an organism resembling *P. pestis* but it was not finally confirmed. This work should be pursued and arrangement for micro-climatic readings of the burrows may yield useful information.

METEOROLOGICAL STUDIES.

The 1949 epidemic phase was unsually prolonged, authentic plague cases being reported till the end of June. Simultaneously, a deviation was also noted in the climatic conditions of the city, although the two observations may not necessarily be correlated. A preliminary study of the seasonal distribution of the plague in different places of India during the last pandemic spreading over 20 years or more has yielded some interesting results. It appears that the plague incidence has two peaks in several areas in India. A more detailed study of the meteorological conditions of Calcutta and, if possible, of other places in India in relation to outbreak of plague has been planned.

REPORT OF THE PLAGUE ADVISORY COMMITTEE.

Held in the Central Secretariat, North Block, New Delhi at 2-30 p.m. on the 18th November, 1949.

MEMBERS PRESENT.

Major-General S. S. Sokhey. (*Chairman*).
Dr. A. M. Francis.
Dr. R. B. Lal.
Dr. Dev Raj Mehta.
Dr. K. V. Venkatraman.
Dr. P. M. Wagle. (*Secretary*).

Dr. C. G. Pandit, Secretary, I. R. F. A. was unable to be present.

VISITORS.

Lieut.-Colonel M. L. Ahuja.
Dr. N. T. Advani.
Colonel S. L. Bhatia.
Dr. T. Chandrasekharaiya.
Lieut.-Colonel A. N. Duggal.
Dr. M. O. T. Iyengar.
Dr. H. Patil. . .
Dr. B. Ananthaswamy Rao.
Dr. R. Subramaniam.
Dr. R. Subramanian.
Dr. Gurkirpal Singh.
Dr. K. S. Viswanathan.

1. Plague researches under the Director, Haffkine Institute, Bombay.

General Sokhey described the work carried out in the Haffkine Institute with the two antibiotics—aureomycin and chloromycetin. In experimental plague infection in white mice he found aureomycin to be almost as effective as streptomycin saving 90 per cent of animals and chloromycetin in same dosage saved 70 per cent of animals. Experiments with larger dosage of chloromycetin are being carried out. No suitable opportunity had occurred this year for clinical field trials.

General Sokhey then described the results obtained with the casein hydrolysate, decolorized by activated charcoal, in the actual production of vaccine. Every broth of the medium before it is used for vaccine production is tested for its growth quality. During the last nine months 38 brews were made and tested, the growth was found to be remarkably constant—varied between 690 million and 880 million organisms per c.c., excepting one brew which gave 1,035 million organisms per c.c. This constancy of the medium permits vaccine of constant protective power to be made.

Dr. Chandrasekharaiya asked if streptomycin could be used as a prophylactic against pneumonic plague and Colonel Ahuja suggested that it would be better if the Committee clarified the question of definition of pneumonic plague.

The Chairman said that pneumonic plague is one which occurs primarily as a respiratory infection and it is highly infectious. Plague pneumonias occurring as a complication to bubonic plague infection are really secondary pneumonias and must not be confused with pneumonic plague. Dr. Wagle said that his experience was that in early stages of primary pneumonias there was no septicæmia while in secondary pneumonias septicæmia was present before the lung infection took place. Dr. Wagle, Dr. Lal and Dr. Francis described cases of pneumonic plague that they had seen in different places. The Chairman said that it was clear that the number of pneumonic cases that occurred in India was very small. In an epidemic of bubonic plague occasionally in a particular locality a few cases of pneumonic plague occur limited to a house or two. General Sokhey personally thought that pneumonic plague was a combined virus and bacterial infection, and that the virus factor made the disease highly infectious.

With regard to the question of the prophylactic use of streptomycin the Chairman preferred sulphonamides to streptomycin as the latter was excreted rapidly from the system.

The Committee considered that as the field work in connection with the trials of various drugs in the treatment of plague was very important and as the only unit which can do this work was in the Haffkine Institute, it was necessary to maintain the staff of the unit and, therefore, recommended the continuation of the inquiry and the grant of Rs. 26,450.

2. Plague inquiry at Coonoor in the Nilgiris district under the Director of Public Health, Madras.

Dr. Francis then described the work done in the Nilgiris with DDT. He said that while plague occurred in 45 out of the 468 villages regularly fumigated with cyanogas once every 3 to 4 months, all the 90 villages treated with DDT only once escaped infection for over 16 months. His observations were that while a single application of 10 per cent DDT was generally sufficient to keep the flea index down below 1 for over 16 months, three to four applications of cyanogas were necessary for the same place during the same period. The cost of DDT application was also comparatively much less than that of cyanogas fumigation.

He had also tried gammexane in one village in the province and found that though treatment with this pulicide had reduced the flea index, the reduction was not comparable to DDT. The experiments were, however, incomplete.

The Chairman suggested the use of water-dispersible DDT for treating walls and floors of houses instead of the powder as it is more economical.

Dr. Mehta said that in the Punjab they had obtained better results with gammexane than with DDT. On Dr. Wagle's inquiring as to how long the residual effect of gammexane had lasted, Dr. Mehta said that in five months the flea index had begun to rise again showing that residual effect of gammexane was less than that of DDT. The Chairman observed that in Italy in Pontine Marshes where DDT had been used systematically as an anti-malarial measure, not only malaria had been controlled, but the general death rate had fallen lower than that of non-malarial areas where no DDT was used. This was due to DDT also controlling fly-borne intestinal infections. He also pointed out that DDT as an anti-plague measure did not affect the rats, it was necessary when carrying out DDT operations, to also deal with rats with the newer rodenticides, not necessarily with the object of controlling plague, but to lessen the economic damage done by rats.

No grant was asked for continuation of this inquiry.

3. Plague inquiry under Dr. R. B. Lal at the All-India Institute of Hygiene & Public Health, Calcutta.

Dr. Lal described the work done in Calcutta. He said that, while 311 cases had occurred in Calcutta this year, in 1948 there were only 173 cases, they were distributed almost in all the wards with the exception of 2 or 3 wards. The cases that had occurred were also of more severe type than in 1948. Mortality was 20·5 per cent excluding the 13 pneumonic cases, and percentage of rats found infected was also higher than last year. In the immunity experiments carried out with the trapped rats, he found the mortality to be approximately 33 per cent. The general flea index was lower than last year but the *cheopis* index was higher but not exceeding one. The few migration experiments done by him were successful, some of the tattooed rats having been recovered from 6 to 8 miles from the original places. Amongst the rats trapped there was relative preponderance of *Rattus rattus.*

The Chairman said that in view of these findings it was evident that *Rattus rattus* and *X-cheopis* were responsible for the spread of plague, and plague in Calcutta was following the same lines as in any other places in India.

Dr. Lal said that his work was handicapped because sanction for the staff, etc., was given for short periods at a time and he did not know how long these sanctions would continue. He desired that the Committee should recommend through the Scientific Advisory Board to the West Bengal Government that the sum of Rs. 16,392 that was sanctioned for the inquiry last year should be made available to him by the Local Government and this would enable him to carry out the work till the end of the present year.

The Chairman observed that the research work which Dr. Lal was doing in connection with the epidemiology of plague in Calcutta was important and should be done in conjunction with the preventive measures adopted in the city and should actually form a part of it. If the work was done without any relation to preventive measures it would be valueless. Therefore, the Scientific Advisory Board should be asked to recommend to the West Bengal Government that not only the sum of Rs. 16,392 should be made available to Dr. Lal now, but that provision should be made in future for his research work in any preventive control scheme which the Provincial Government would adopt.

Dr. Lal wanted the Advisory Committee to clearly understand that he needed a provision for this inquiry to be made for a period of five years at least and, if such a provision could not be made, he did not want any provision to be made for the next year alone. He did not think he could do any justice to the inquiry in a shorter period than five years.

In view of this clear statement and the Chairman's view that this inquiry should be linked with the preventive work being conducted in Calcutta, the Committee recommended that the Scientific Advisory Board should urge on the West Bengal Government to make provision for this inquiry out of its funds allotted for control work, as this inquiry would make the control measures much more effective. No grant was recommended for this inquiry from the Indian Research Fund Association.

4. Inquiry into the recrudescence of bubonic plague in the East Punjab under the Director of Health Services, East Punjab, Kasauli.

The proposal for the grant for this inquiry was then considered. Dr. Mehta said that it was necessary to obtain certain basic facts regarding the density of population, species and incidence of plague infection in rats in the endemic plague areas in the Punjab, as also the species of fleas, their index, etc. in those areas at different seasons of the year with a view to enable him to adopt the control measures in time. He proposed to carry out this work in 15 selected places in endemic areas.

The Chairman said that this work really formed part of routine control measures that should be adopted by the Local Government and, therefore, this inquiry should be sponsored by them.

Dr. Mehta said that the Punjab Government was already contributing a sum of about Rs. 20,000 and he was approaching the Indian Research Fund Association to give him primarily some specialized staff.

Dr. Lal was in favour of giving him a reduced staff. Some discussions followed and it was ultimately agreed that a reduced staff should be recommended and a sum of Rs. 10,060 be sanctioned for this inquiry.

2. Inquiry to study culture media of *B. pestis* for growth under optimal conditions for antigenic and protective purposes under Dr. C. V. Natarajan.

Dr. Chandrasekharaiya said that it was necessary to do this work with a view to preparing a vaccine which would not give toxic reactions. The Chairman said that already a very potent and atoxic vaccine was being prepared in the Haffkine Institute, and it seemed that Bangalore had not adopted this advanced technique. If any research is to be done it should start from the point to which the manufacture of plague vaccine had been taken by the Haffkine Institute and advances made beyond that point.

Drs. Wagle and Lal said that the programme given covered so many items that Dr. Natarajan would never be able to do these all at one time.

The Chairman said that it would be advisable for Dr. Natarajan to start the work on one or two aspects of the problem particularly with regard to the nutritional requirement of the plague bacillus, and then after doing this work for a year, he should present his results at the next year's meeting and then, on the basis of the results obtained, ask for a grant.

The Committee agreed to this suggestion and no grant was recommended for this inquiry this year.

CLINICAL RESEARCH.

1. Clinical research unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

The plan of the work mentioned in the last report was modified to some extent.

After correspondence with Dr. Lansing at Washington University, St. Louis, who has worked on changes in calcium content of mouse epidermis during carcinogenesis, a technique has been developed for the determination of calcium and its diffusible and non-diffusible forms. Many difficulties were encountered in adapting the recommended method to liver-tissue, with the equipment available in the country. We are grateful to Dr. V. N. Patwardhan for his advice in overcoming certain technical difficulties and the setting up of a simplified ultra-filtration apparatus. The standardization of the technique has involved much labour and time and it may now be possible to undertake a quantitative estimation of free and bound calcium in liver-tissue with reasonable accuracy.

It was not possible to take up the work on two enzymes, ketoglutanic oxidase and pyruvic oxidase, as the required reagents are still not available. However, the technique of paper-partition chromatography using standard amino-acid solutions has been worked out for the determination of relative concentrations of amino-acids in liver-tissue. The evaluation of the chromatographs has to be carried out on the basis of protein and nitrogen content of tissue. These estimations, therefore, are being carried out on normal rat liver.

Investigations on hospital patients.—The studies on urinary excretion of vitamin B were extended to patients suffering from cancer of cervix uteri and also to normal women of corresponding age. Twelve patients and four normals were studied. The investigations on one subject usually takes a week for completion. During the course of investigations, the creatinine values found for 24-hour urine samples from the same subject, showed fluctuations much beyond experimental errors. This suggested that the instructions given to a test person were not being strictly followed out in the collection of the urine. It was thus impossible to obtain exactly '24-hour' urine sample and the work had to be postponed.

2. The neuropathological unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

The period of nine months was spent in training the staff and in obtaining equipment necessary for the neuropathological unit. The following specialized techniques required for histological study of nervous system have been developed and practiced.—

1. *Neuropathological techniques :*
   - A. Bielschowsky—for axis cylinders
   - B. Gross Schultz—Peripheral axis cylinders
   - C. Denny-Brown—Axis cylinders, peripheral
   - D. Globus-Cajal—Astroglia
   - E. Cajal—Astroglia

F. Hortega—Oligodendroglia
G. Pickworth—Angioarchitecture
H. Spielmeyer—Myelinsheets
I. Fat stain after gelatine embedding.

2. *Paraffin techniques :*

A. Holzer—Gliofibres
B. Myelin
C. Holmes—Axis cylinders

It is expected that this part of the training would be completed before the end of this year.

The histopathology of cerebral malaria has been carefully worked out. This investigation has yielded very interesting results which are being prepared for publication.

3. Inquiry on the role of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay.

1. EXPERIMENTAL HEPATIC CIRRHOSIS.

The influence of tocopherol on the production of massive hepatic necrosis in rats.—In 1944 Himsworth and Glymn claimed to have produced massive hepatic necrosis in rats by low-protein diets and the factor in protein, lack of which caused necrosis was subsequently (1945) shown by them to be cystine. Since then it, however, became clear that, even when similar diets are used, there are discrepancies between the results obtained in the different laboratories. Radhakrishna Rao (*Nature*, p. 161, 1948) enumerated the different factors which might be responsible for these discrepancies. In the meantime, experiments of Schwartz (1944) György (1947) seem to have indicated the importance of tocopherol content of the dietary fat on the incidence of liver necrosis in animals. In order to throw further light on this point, the following experiments have been planned and are in progress :—

(*a*) *Prevention of necrosis of the liver of rats by tocopherol :* Two groups of rats have been placed on the yeast-protein diet, similar in composition to that which Himsworth found to be most effective in producing massive hepatic necrosis. One group is being given a supplement of 15 mg. to 20 mg. of tocopherol and the other group serves as control and no tocopherol is given. The experiment is in progress.

(*b*) *The effect of pre-experimental diet on the development of massive hepatic necrosis in rats :* Two groups of rats on weaning (each group containing 18 rats) have been put on a diet containing alcohol-extracted casein, yeast and lard (as a dietary fat) in order that the diet is deficient in tocopherol. The diet of one group was supplemented with tocopherol. After 120 days on these diets, both groups have been placed on yeast-protein diet without the supplement of tocopherol. It is now nearly 40 days since the commencement of the necrogenic diet. It will be necessary to continue this experiment for longer periods and on completion the detailed results will be submitted.

(*c*) *Comparative effect of lard and hydrogenated vegetable fat (dalda) on the production of massive liver necrosis in rats fed on low-protein diets* : In our previous experiments on dietary hepatic cirrhosis in animals, we have used mostly hydrogenated vegetable fat (dalda) as the fat moiety of the diet. Vegetable fats are fairly rich in tocopherol, while lard is devoid of it. This experiment has, therefore, been started to study the relationship between the incidence of liver necrosis and the type of dietary fat.

2. CLINICAL LABORATORY WORK.

Total phospho-lipoid, choline-containing and non-choline containing phospho-lipoids in the serum of normal human subjects and in patients with cirrhosis of liver.—Three types of phospho-lipiods are recognized at the present time, namely, lecithin, cephalin and sphingomyelin. The biochemical importance of these substances as a group is well established. Phospho-lipoids are intermediary products of fat metabolism. They also play an essential role in the structural make-up of cells, particularly cell membranes. Cephalin and sphingomyelin are considered as structural type of phospho-lipoid, because they together constitute a large part of the phospho-lipoids present in cells and stroma, while lecithin is believed to be the vehicle most intimately concerned with the transport of fatty acids in the animal body.

The development of micro-methods for the determination of individual phospho-lipoid lecithin, cephalin and sphingomyelin has made possible in recent years investigations concerning the physiology and pathology of phospho-lipoid metabolism and the phospho-lipoid distribution in the blood. The methods are similar in that in each case a quantitative determination of choline is made. Lecithin and sphingomyelin contain approximately equal amounts of this base, whereas none is present in cephalin. Thus, by determining total phospho-lipoid and choline, the cephalin content can be calculated.

A study of the changes in the distribution of the phospho-lipoids of blood in cirrhosis of liver and in other allied liver diseases in man is of particular importance and interest in view of the active part played by the liver in the metabolism of fat. Liver is the most important site where the phosphorilation of the fatty acids takes place and recent isotopic studies have shown that the turn-over of phospho-lipoids occurs at a more rapid rate in liver than in any other organ. Recently, Chaikoff and his co-workers (1948) have shown that the plasma phospho-lipoids are manufactured in the liver.

These results indicate that a study of the distribution of the phospho-lipoids of the blood-serum of patients with cirrhosis of liver, may be of clinical significance from the point of view of the prognosis of the disease or of the liver function.

Extraction and precipitation of serum phospho-lipoids.—One part of serum to 20 parts (by volume) of Bloor's (3 : 1) alcohol-ether mixture was us for the extraction of serum phospho-lipoids. The alcohol-ether extrac was completely evaporated under reduced pressure and low temperatur and re-extracted with petroleum-ether. Phospho-lipoids were precipitate from petroleum-ether solution by the use of acetone and $MgCl_2$ according to Bloor and dissolved in (1 : 1) methyl-alcohol and ether.

Estimation of lipid phosphorous.—For the determination of total phospholipoids, lipid P was estimated in an aliquiot of the solution by the Fiske and Subbarow's colorimetric method and the value of lipid P in mg. per 100 c.c. serum multiplied by 25, gave the value of total phospho-lipoids in mg. per 100 c.c. serum.

The estimation of choline in phospho-lipoids by the micro-methods of Erickson et al. (1940).—The procedure described by Erickson (1940) for the micro-estimation of choline in phospho-lipoids is very simple and the accuracy of the method has been tested by the following recovery experiments (Table I) :—

TABLE I

Recovery of choline.

Choline standard (choline added as standard choline-chloride solution).	Choline present, mg.	Choline added, mg.	Total choline, mg.	Total received :	
				Mg.	Per cent.
	0·039		0·039	0·033	84
	0·040		0.040	0·040	100
	0·040		0·040	0·038	92
	0·078		0·078	0·068	86
	0·080		0·080	0·072	91
	0·117		0·117	0·102	87
	0·121		0·121	0·113	93
	0·156		0·156	0·151	97
	0·200		0·200	0·180	89
Serum extract ..	0·027	0·078	0·105	0·090	86
	0·052	0·078	0·130	0·124	95
	0·025	0·119	0·144	0·144	100

From our experience, we have found that choline within the range from 0·04 mg. to 0·16 mg. can be accurately estimated by this method provided the procedure is properly followed.

Relation of total choline and total protein in the serum of normal human subjects.—From 13 normal persons total serum-protein and choline content were estimated to find if there is any correlation between these two and the results are shown in Table II. Total protein in serum is expressed as g./100 c.c. serum and the results of choline are expressed as mg./100 c.c. serum.

The choline content of serum varied within 15 mg. to 25 mg. per 100 c.c. serum, with an average of 20·1 mg. and there appears to be no relation between total serum-protein concentration and choline, because at the same level of total serum-protein, different values of choline are obtained.

TABLE II.

Total protein and choline content per 100 c.c. serum in normal human subjects.

Sample number.	Total protein, g./100 c.c.	Choline, mg./100 c.c.
1	7·17	16·0
2	6·79	15·5
3	7·19	18·0
4	7·16	20·5
5	6·64	16·0
6	7·25	25.0
7	7·49	20·5
8	6·61	22·7
9	7·16	23·5
10	6·80	16.0
11	7·43	22.0
12	6·90	21.1
13	6·78	24.2
Totals ..	7·03	20·1

Total phospho-lipoid, choline-phospo-lipoid and non-choline-phospo-lipoid in normal human serum.—The phospho-lipoid analysis of 11 normal human sera of both sexes is shown in Table III :—

TABLE III.

Phospho-lipoid analysis of normal human serum mg. per 100 c.c. serum.

Subject number.	Sex.	Age.	Lipid P.	Choline.	Choline : P ratio.	Total phospho-lipoid.	Choline-phospho-lipoid.	Cephalin.
1	M	33	7·83	26·95	0·882	195·8	172·7	23·1
2	M	26	5·996	17·72	0·76	149·9	113·48	36·42
3	M	26	7·09	26·58	0·96	177·25	170·16	7·09
4	M	28	6·15	20·98	0·88	153·74	138·38	19·37
5	M	23	5·844	18·94	0·93	146·10	121·26	24·84
6	M	29	5·49	19·82	0·925	137·20	126·91	10·29
7	F	26	6·16	19·44	0·81	154·0	124·59	29·41
8	F	22	7·24	24·69	0·874	181·0	158·19	29·81
9	M	28	4·74	20·43	1·100	118·0	118·0	0
10	M	20	3·57	11·26	0·815	89·25	72·74	16·51
11	M	27	4·45	18·90	1·08	111·25	111·25	0
Average ...		3	5·87	20·52	0·901	146·68	129·79	17·9

In all the above normal persons, blood samples were drawn before breakfast for the phospho-lipoid analysis of the serum. The average value of the lipid P in 11 cases was found to be 5·87 mg. with a range of 3·57 mg. to 7·83 mg. per 100 c.c. serum and is somewhat below the standard range of 8 mg. to 10 mg./100 c.c. serum, as determined in normal persons of Western countries. The average choline content of the serum in 11 cases was found to be 20·52 mg. per 100 c.c. serum and these results are found to be quite in agreement with those reported by Thannhauser (1939) in serum, Erickson (1940) in plasma, Artom (1941) in plasma and Marenzi and Cardini (1943) in plasma. In six samples of normal human serum Thannhauser found choline within a range of 12·5 mg. to 33·6 mg. per 100 c.c. serum with an average of 19·3 mg. In 16 samples of normal human plasma at fasting, Artom found choline within a range of 15·5 mg. to 23·5 mg./100 c.c. plasma with an average of 18·2 mg. Erickson found on an average 20 mg. per 100 c.c. plasma from four samples of normal human plasma. In 11 samples of normal human serum, the total phospho-lipoid was found within a range of 89 mg. to 196 mg. per 100 c.c. serum with an average of 146 mg., and was somewhat below the average Western standard. The above observations agree with those of Entemann *et al.* that the serum phospho-lipoids are predominantly choline-containing type. Non-choline phospho-lipoid (cephalin) forms only a small fraction of the total phospho-lipoids present in serum.

Phospho-lipoid partition of serum of patients with cirrhosis of the liver.— The results of the analysis of serum phospho-lipoids of patients with cirrhosis of the liver are shown in Table IV:—

TABLE IV.

Serum phospho-lipoids in cirrhosis of the liver (mg./100 c.c. serum).

Name.	Accompanying symptoms.	Lipid P.	Choline.	Choline: P ratio.	Total phospho-lipoid.	Choline-containing phospho-lipoid.	Cephalin.
C.J.	Ascites for 3 months.	4·73	10·96	0·593	118·25	70·12	48·13
P.R.	Ascites for 2 months.	4·41	11·56	0·67	110·25	73·87	36·38
A.H.	Ascites	4·84	15·90	0·84	121·0	101·64	19·36
K.G.	Ascites	3·49	9·54	0·70	87·25	61·07	26·18
B. Y.	Distension of abdomen.	4·54	14·36	0·81	113·38	91·95	21·43
M.D.	Ascites for 4½ months.	3·57	7·02	0·504	89·25	44·98	44·27
B.P.	Distension of abodmen for 3 months.	4·89	12·5	0·65	122·3	79·5	42·8
S.B.	Ascites	5·71	16·52	0·74	142·8	105·8	37·0
	Average ...	4·52	12·3	0·69	113·06	78·6	34·4

A marked reduction of the total phospho-lipoids, and particularly choline-containing phospho-lipoids, has been found to be an important feature of all the cases of cirrhosis of the liver with ascites. Cephalin content of the serum, on the other hand, shows some increase over the normal values.

Table V shows the results on the increase of the total phospho-lipoids and choline-containing phospho-lipoids of the serum of patients with treatment and partial clinical improvement :—

TABLE V.

Phospho-lipoid partition of serum in cirrhosis of the liver on treatment.

Case number.	Name.	Date.	Total phospho-lipoid mg./100 c.c. serum.	Choline-containing phospho-lipoid mg./100 c.c. serum.	Cephalin, mg./100 c.c. serum.
1	A.S.	17-1-49	133·3	108·7	24·6
		3-2-49	139·5	108·3	31·2
		7-3-49	128·5	127·2	1·3
2	B.Y.	3-1-49	113·5	91·94	21·56
		19-2-49	125·0	87·50	37·5
		7-3-49	166·8	150·10	16·65
		23-3-49	186·0	153·0	33·0

It will be seen that with treatment there is an increase in the choline-containing phospho-lipoids. The results are very suggestive and further work on this line may bring about some useful information.

4. Clinical and biochemical investigations on certain aspects of infantile hepatic cirrhosis under Professors V. Subrahmanyan and Dr. V. R. Naidu at the Central Food Technological Research Institute, Mysore and Sri Krishnarajendra Hospital, Mysore.

During the year under report a total of 107 cases was studied and treated. Most of the cases were treated as out-patients but were under close observation of the Medical Assistant, who also visited them in their homes whenever necessary. In one of the wards of the Sri Krishnarajendra Hospital, Mysore a few beds had been set apart exclusively for cases of infantile hepatic cirrhosis. A few select cases were hospitalized for short periods for special study and treatment.

In continuation of the work reported previously the investigation comprised : (1) the *therapeutic action* of choline chloride as compared with that of a pepsin-proteolysed extract of the liver and spleen supplemented with a small amount of choline chloride, (2) comparison of several *liver-function tests* with particular reference to their applicability for diagnosis as well as prognosis in this disease, (3) *histopathology of the liver* as revealed by biopsy specimen obtained at different stages of the disease and (4) certain *biochemical studies.*

The following account summarizes the results of these studies and indicate the lines of further work :—

1. THERAPEUTIC STUDIES.

The classification of the disease into several pathological stages with the related symptomatology as also the criteria of clinical improvement and cure have all been described in detail in previous reports. Liver-function tests, particularly the thymol-turbidity test, have been used as additional confirmatory aids to clinical symptoms in the initial diagnosis and assessment of the exact pathological stage as well as for prognosis under treatment.

A group of 64 cases was treated with choline chloride. They received pure choline chloride in the form of a faintly acid (0·05 NHCl) 25 per cent solution of the salt in 10 per cent alcohol, the mild acidity preventing hydrolytic degradation of the salt and the alcohol protecting the solution against bacterial contamination. The dosage was 250 mg. of choline chloride twice daily, i.e. 500 mg. per day per child. The bitter salt was rendered as palatable as possible by administering it along with sugar or a little honey.

The rest 43 cases were treated with a pepsin-proteolysed extract of the liver and spleen prepared as described in earlier report and supplemented with 10 mg. per c.c. of choline chloride. Two teaspoonfuls twice a day were prescribed for each child.

An adequate supply of vitamins was ensured in all cases by the oral administration of B-complex preparations supplemented with vitamin C and adexolin.

Secondary complaints, such as constipation, temperature, anæmia, etc., were treated symptomatically. Bi-weekly injections of liver extract with vitamins of the B-complex and vitamin C were given to counteract anæmia. Diuretics were given orally (diuretic mixture, calcium-diuretin tablets, liquid extract of punarnava and kalmegh) and parenterally (esidrone, aminophylline) in the advanced stages of the disease in order to relieve anuria and consequent œdema and ascites. Although these drugs increased the frequency of urination in the first instance, there was no marked increase in the quantity of urine voided and later there was a relapse of severe anuria. Paracentesis was resorted to in a few advanced cases with a view to mitigate the respiratory and other distress due to the accumulation of fluid in the abdomen. There was, however, little permanent benefit from this measure.

The dietary of the children under treatment was restricted to foods with very little fat, e.g. separated milk, rice with soups of greens, butter-milk, fruit juice and barley water. Glucose and sugar were allowed liberally. An intake of about 1,000 cals. per child per day was ensured.

The clinical results of the two courses of treatment are given in Tables I and II :—

TABLE I.

Treatment with choline chloride only.

Pathological stage.	Total number treated.	Number cured.	Per cent cured.	Period of treatment for effecting cure, in weeks.
I ..	40	40	100	3–5
II-A ..	81	81	100	6–12
II-B ..	40	23	57·5	9–16
III ..	26	..	0	..

TABLE II.

Treatment with pepsin-proteolysed liver and spleen extract supplemented with choline chloride.

Pathological stage.	Total number treated.	Number cured.	Per cent cured.	Period of treatment for effecting cure, in weeks.
I ..	38	38	100	2–4
II-A .	47	47	100	3–6
II-B .	33	21	63·6	8-10
III .	28	..	0	..

For the sake of proper comparison the results in the present study have been combined with those obtained previously in the respective groups.

A case has been considered clinically cured when there is no palpable enlargement of the liver and spleen, the clinical symptoms have been completely relieved and the general condition has been restored to normal.

Cases which proved resistant to treatment with choline chloride alone in the II-B stage received a supplement of the liver-spleen hydrolysate as in the other group, after a trial period of six weeks or earlier in case any serious symptoms developed. In reckoning the 'per cent cured' in the above group, however, only those which were cured by the administration of choline chloride alone have been taken into consideration.

In all recovered cases treatment has been continued for periods of 8 to 12 weeks after complete recovery. A number of these cases has been followed up for quite long periods after treatment was discontinued.

The general observation that the glandular hydrolysate supplemented with a small amount of choline chloride is superior in therapeutic effect to three times that quantity of choline chloride administered alone has been confirmed. The pace of clinical recovery is slower in the choline-treated cases. The cholagogic action of choline is milder. The fat content of the stools is reduced to normal in 2 to 3 weeks in the choline-treated group, whilst this happens in about a week in the other group. Choline chloride does not appear to stimulate appetite until after a long period of treatment. On the other hand, stimulation of gastric secretion and appetite have been found to be an important property of the glandular extract. The glandular extract exerts a marked hæmatopoietic action in the early stages of the disease. In the choline-treated cases liver-extract therapy is invariably needed for counteracting anæmia even in the early stages of the disease.

2. Liver-function tests.

The comparative study of the thymol turbidity, the cephalin-cholesterol flocculation and the bromosulphalein-retention tests of liver function at different stages of the disease were continued. The details of the tests are described in last year's report.

In confirmation of our earlier findings, it has been found that the thymol turbidity and the bromosulphatein-retention tests run more nearly parallel to the pathological condition as determined by clinical symptoms. The degree of flocculation of the cephalin-cholesterol emulsion varies very irregularly with the clinically assessed pathological condition. In view of the comparative simplicity and convenience of procedure we have adopted the thymol-turbidity test as a routine test both for initial diagnosis and for prognosis under treatment.

In the thymol-turbidity test the turbidity decreases with the advance of the pathological condition : Stage I—5 to 7 units, stage II-A—4 to 6 units, stage II-B—3 to 4 units and stage III—2 to 3 units. A decrease in the turbidity with remission of concomitant clinical symptoms is indicative of recovery. A persistent low reading (of 3 and less) denotes unfavourable prognosis. In conjunction with clinical symptoms the test can be employed for both diagnostic and prognostic purposes.

Further studies on these tests in this and other hepatic diseases, with a view to correlate the different tests with the type and extent of hepatic damage as revealed by histological examination of biopsy specimens of the liver, are in progress.

3. Histopathology of the liver : biopsies.

So far liver biopsies have been done in 29 cases, i.e. ten cases of the II-A stage, eleven of the II-B stage and eight of the III stage.

The biopsy technique was essentially the same as that described by Sheila Sherlock (*Lancet*, **249**, p. 397, 1945).

Every precaution was taken to guard against the danger of hæmorrhage consequent to the operation. After selecting a case for biopsy, the patient's blood-coagulation time was noted and 10 mg. of vitamin K (kapilin, K-vimin) was administered orally 3 to 4 times daily for 2 to 4 days prior to the day of the operation. On the day of the biopsy the patient was given 20 mg. of vitamin K in two equally divided doses 2 to 4 hours before the operation. One injection of 2·5 c.c. of coagulum CIBA was given half an hour before and another half an hour after the biopsy. Biopsies were done only on cases in which the blood-coagulation time was within reasonable limits.

The operation was performed under local anæsthesia produced by injecting 10 c.c. of a one per cent solution of plainocain. Tincture iodine was used as a surface antiseptic.

The site of the operation was a point on the lateral margin of the right rectus half-an-inch below the tip of the tenth rib. All biopsies were done on the right lobe of the liver.

After the operation a tincture-benzoin seal was applied to the site of operation and securely fastened in position by leucoplast bands.

The post operative reaction of the patients was carefully watched. For 3 to 4 days after the operation 10 mg. of vitamin-K were given 3 to 4 times daily. Only liquid diet was allowed during this period. Any secondary reaction, such as temperature, etc., were treated symptomatically. So far no untoward reactions have been observed in any of the cases as a result of the biopsy.

The Vim-Silvermann needle was used for the biopsy. The specimen usually obtained with this needle was ½″ to ¾″ long and 1 mm. to 2 mm. thick and weighed between 15 mg. and 20 mg.

The biopsy specimens were fixed in Bouin's fluid run through the usual procedures and finally embedded in paraffin. Sections 3μ to 5μ thick were then cut and stained in the usual manner with hæmatoxylin-eosin, iron hæmatoxylin, Van Gieson and other stains.

The histopathological findings as revealed by the examination of the sections of biopsy specimens are summarized in Tables III and IV.—

TABLE III.

Microscopic features.

Stage :—	II-A.	II-B.	III.
(1) Size of the liver	2 to 3 fingers below the costal margin.	2 to 4 fingers below the costal margin.	1½ to 2½ fingers below the costal margin on the right side and 2 to 2½ fingers above the umbilicus on the left.
(2) Colour (of biopsy specimen).	Brownish	Grey	Greenish white.
(3) Involvement	Both lobes uniformly enlarged.		
(4) Surface (as palpated).	Smooth	Finely granular	Coarsely granular.
(5) Margin	Entire (not broken).	Thin and sharp.	
(6) Consistency	Soft, cuts easily with the biopsy needle.	Hard and firm.	
(7) Bile staining	..	..	Present.

TABLE IV.

Microscopic features.

Stage :—	II-A.	II-B.	III.
(1) Architectural pattern.	Not lost. Here and there normal liver lobules bearing a proper relation to the portal tract and central vein are seen.	Markedly changed.	Completely distorted.
(2) Parenchyma (nodular character).	Pseudo-lobules are not seen.	Pseudo-lobules are seen but are not so well defined as in the stage III.	Pseudo-lobulation marked. Regenerative activity well seen in cases which have been under treatment.
(3) Necrosis	Diffuse, in small irregular patches.	Diffuse but more marked.	As in II-B.
	The necrotic lesions are seen more towards the centre than the peripheri of the lobules. In the II-B and III stages necrotic areas are surrounded by rings of atrophic liver cells.		
(4) Hyalin	Seen sparsely distributed.	Definite hyalinization seen in 5 to 10 per cent of the cells.	
(5) Liver cell structure	Some are normal, others distended and vacuolated, containing lipoid material.	General picture same as in the previous stage. More hypertrophic cells seen.	A large number of cells is distended and vacuolated. A few multinucleuated cells with 1 to 2 deep-staining nucleoli are seen.
		In the II-B and III stages atrophic changes are seen along the peripheri of the regenerating nodules.	
(6) Fatty metamorphosis.	Fatty change mild, scattered or patchy.	Present but not very marked.	
	Almost all of the cases had been treated with 500 mg. choline chloride per day for periods varying from 2 to 8 weeks prior to biopsy.		

Table IV.—(*Concld.*)

Stage :—	II-A	II-B	III
(7) Connective tissue.	Fibroblastic activity poor.	Definite fibrotic tissue seen penetrating the pseudo-lobules.	Bands of connective tissue with varying degrees of collagenous fibres seen, both peri-cellular and peri-lobular in distribution.
(8) Bile ducts	No proliferation seen.	Proliferation present in varying degrees.	Both condensation and proliferation seen, but not extensive.
(9) Lymphatics	Lymph spaces (spaces of Desse) are enlarged here and there.	Spaces of Desse more numerous among the atrophic and regenerating cells.	
(10) Signs of inflammation. Cellular infiltration			
(*a*) of the parenchyma.	*Nil*	Present	Present and marked.
(*b*) of the connective tissue	*Nil*	..	Mostly lymphocytic (mononuclears).
(11) Vascular pattern	No alteration	Altered No evidence of endophelbites.	Altered.
(12) Hæmosiderosis	..	..	..
(13) Regeneration	Not marked	Present	Present.
(14) Bile pigment	Absent	Absent	Present when jaundice is marked.

Cultures of biopsy specimens did not show the presence of any pathogenic or other organisms excepting in two cases where growth of *S. aureus* was seen, most probably due to extraneous contamination.

4. BIOCHEMICAL STUDIES.

(*a*) *The effect of whole milk diets on the composition and structure of liver tissue.*—Our own observations as well as those of others have shown that the incidence of infantile hepatic cirrhosis is highest among the vegetarian communities. It has also been found that the large majority of cases are those who have been fed almost exclusively on cow's milk from birth or some time later up to weaning. The incidence is maximum during the critical period of change-over from the milk to the poor protein, high carbohydrate diet. These general observations are suggestive that some inherent nutritional defects and also the immediate dietary history of the children have an important bearing on the ætiology of the disease.

Cow's milk, although an infant food par excellence, would constitute a relatively high fat diet when fed exclusively and under conditions in which its protein moiety is not properly utilized. In case of defective metabolism of the protein, the fat of the milk would also not be utilized in the normal manner and may get deposited in the liver. Certain vitamin and other deficiencies or some abnormal constituents in the milk may lead to a similar result. Persistent infiltration of fat into the liver, it has been experimentally demonstrated, leads ultimately to cirrhosis and its clinical sequelæ.

A low capacity to utilize protein and fat may be inherent in a child by reason of the predecessors having consumed for generations a low-protein, high-carbohydrate diet, such as the poor rice diet. If to such a child a fat-rich food, as cow's milk, is given exclusively it probably leads to accumulation of fat in the liver. The peculiar physiological changes occurring during the critical growth period of the child, concomitant dietary deficiencies and also infections may all act as aggravating factors producing in the end cirrhotic changes in the liver. If the conditions under which milk feeding leads to fatty infiltration of the liver could be determined experimentally, then one could find out how far such conditions obtain in the children suffering from hepatic cirrhosis. This would throw much light on an important ætiological factor of the disease.

In view of the above considerations it was thought necessary to ascertain in the first instance the effect of feeding whole milk to growing experimental animals on the histological structure and chemical composition of the livers. Accordingly the following experiment was started :—

Experiment : A group of 24 young albino rats (six weeks old) were put on a diet of boiled whole milk obtained from a local dairy. The average composition of the milk was 2·5 to 3 per cent protein and 3 to 3·5 per cent fat. The milk was given *ad lib.* In addition to the milk each rat was given 5 g. of corn starch to provide some solid and supply adequately the caloric requirement.

At the end of four weeks six of the rats were exsanguinated through the abdominal aorta and the liver excised under amytal anæsthesia. A portion of the liver was examined by routine histological methods and the rest analysed for its water, protein and fat contents. The analytical results are given in Table V. The average values are expressed on the basis of 100 g. of body-weight :—

TABLE V.

Dietary group.	Dry liver-weight, g.	Water, g.	Protein-N, g.	Fat, g.
Milk-fed	1·03	2·45	0·110	0·196
Controls	1·20	2·40	0·113	0·121

The results show that on the average the fat content of the livers of the milk-fed animals is nearly twice that of the controls fed on an adequate stock diet.

Histologically the liver sections have shown only infiltration of finely dispersed fat particles into the parenchymal cells.

The experiment is being continued. It is proposed to follow up the chemical and histological changes in the livers of the experimental animals by periodically sacrificing a definite number and examining their livers.

(*b*) *Plasma cholesterol and lipid phosphorus.*—These were determined in a number of cases and are given in Table VI :—

TABLE VI.

Pathological stage.	Number of cases.	Cholesterol.	Lipid phosphorus.
		mg. per 100 c.c. plasma.	
I ..	7	180–240	3·5–6·8
II-A ..	12	160–310	3·0–6·0
II-B ..	8	200–450	3·4–5·2
III ..	6	350–580	2·5–4·0

The results indicate that the plasma-cholesterol level is elevated particularly in the advanced stages and that of lipid phosphorus lowered in this disease.

(*c*) *Partition of phosphorus in plasma.*—The partition of phosphorus (total, inorganic, ester and lipid) compounds have been determined in eight cases according to procedures described in " Micro-Analysis in Medical Biochemistry " by King (1947). The results are given in Table VII:—

TABLE VII.

Pathological stage.	Number of cases.	Phosphorus : mg./100 c.c. plasma.			
		Total.	Inorganic.	Ester.	Lipid.
I ..	1	7·8	4·3	0·3	3·2
II-A ..	3	7·5	4·0	0·2	3·0
II-B ..	3	6·6	2·2	0·2	4·0
III ..	1	6·0	1·8	0·2	3·8

These studies are being continued.

In addition to the above, we have commenced a study of the content of amino-acids and other non-protein nitrogenous compounds in the blood. Methods are being standardized for the determination of serum-choline concentration at different stages of the disease. We hope to be able to complete a major part of the study on blood chemistry during the current year itself.

DISCUSSION.

The clinical results obtained so far have shown that the pepsin-proteolysed extract of the liver and spleen supplemented with a small amount of choline chloride is superior in therapeutic effect to thrice that quantity of choline chloride administered by itself. In a previous series of clinical trials it has been found that supplementation of choline chloride and also casein hydrolysate to the glandular extract evoked the very best of clinical responses (85 per cent cured in the II-B stage) in the advanced stages of the disease. Individually the glandular extract choline chloride and casein hydrolysate were found to be effective only in the early stages of the disease.

Cirrhosis of the liver is a complex syndrome in which there is a gradual failure of the regenerative metabolic and other functions of the liver. In its therapeutic management there is every need to provide in a consolidated form as many of the active principles as possible which would stimulate the regenerative and functional activities of the liver. This is the rationale for the use of ' crude ' whole-liver preparations in the treatment of liver cirrhosis. Instead of a crude extract of water-soluble extractives of the liver, we employed a pepsin-proteolysed extract with a view to render the active principles more readily available than we considered would otherwise be possible under the conditions of gastro-intestinal disturbance obtaining in infantile hepatic cirrhosis.

It is likely, however, that all the necessary active factors of the liver have not been brought into or sufficiently well preserved in the glandular extract. The pepsin-proteolysed extract of the liver and spleen, we have found, deteriorates slowly on standing at room temperature and for the best and most beneficial effects only preparations which have not stood for more than four weeks should be used. Attempts to concentrate the extract by vacuum distillation or drying *in vacuo* and also extensive hydrolysis of the glandular protein with pancreatic trypsin have all resulted in unsatisfactory products. The procedure adopted for hydrolysis may also have been deleterious to some of the active principles. Perhaps, preliminary enzyme digestion may not be necessary for successful extraction of the active principles from the liver. On the basis of clinical experience we have gained the impression that the extract we have used contains certain active principles which should be better preserved and concentrated and, if necessary, augmented with other factors for more effective therapeutic treatment in the advanced stages of the disease, especially in the II-B stage wherein an appreciable proportion of cases have proved resistant to treatment.

The immediate preparative work should, therefore, be concentrated on other methods of extraction and preservation of the active principles of the liver. A possible method of concentration and preservation that suggests readily is the freeze-drying of such extracts. The therapeutic efficacy of the preparations should be studied both when administered alone and as supplemented with lipotropic factors, such as choline chloride, and protein factors, such as casein hydrolysate.

Dietetics form an important adjunct to therapeutic treatment particularly in liver cirrhosis where there is loss of apetite and the utilization of the dietary constituents is impaired. In infantile hepatic cirrhosis there is sufficient evidence suggestive of the poor digestion and utilization of the protein and fat in the diet. Therefore, fat should be either completely eliminated or minimized in the diet and in addition the digestion and utilization of protein and carbohydrate assisted. Carbohydrate could be given in the partly pre-digested form as in some of the commercial-processed infant foods. Protein digestion could be assisted by the provision of gastric and pancreatic enzyme preparations. These may be administered either before feeding or along with the food. These may also be allowed to act on the food preparations for some time and then the partly pre-digested food along with the enzyme preparations given to the child. The response of patients to such dietetic management and its influence on the therapeutic treatment of the disease would form an important part of further clinical studies.

The liver-biopsy studies should be continued and extended to the very early stage of the disease, using different type-staining methods to obtain a more detailed and exact picture of the histological changes in the liver at different stages of the disease.

The comparison of the several liver-function tests at different stages of the disease should be continued. The tests should be correlated with the histological findings as revealed by biopsy specimens to determine how far they are indicative of the extent of hepatic damage.

The experimental investigations on the effect of whole-milk diets on the composition and structure of the liver should be continued and further extended in view of the interesting findings already made. Using whole milk (milk powder) as the basal diet the effect of vitamin and mineral supplements should be ascertained. It is important from the practical standpoint to study the alterations in the liver that take place when experimental animals are changed over from whole milk to poor rice diets. The influence of prolonged feeding on a poor rice diet on the subsequent utilization of the protein and fat of whole milk as revealed by balance studies and the protein and fat content of the liver would also yield valuable data bearing on the above question. In addition to studying the composition and structure, liver-function tests should be carried out in all cases to find out any impairment in the functional activity of the liver.

The biochemical studies have so far shown that the changes in the blood constituents observed are those generally characteristic of hepatic dysfunction. A more complete analysis of the constituents in the blood at different stages of the disease is essential before one can define the changes specifically pathognomonic of this disease.

That a lipotropic factor, i.e. choline chloride and casein hydrolysate are therapeutically effective in the early stages of the disease, is suggestive that the initial lesion of the liver in this disease arises from failure to mobilize fat from the liver. The fat that has accrued in the liver may originate from exogenous (dietary) or endogenous sources. The failure in fat metabolism in the liver may be due directly to a lack of choline in the diet or to defective biosynthesis owing to some abnormal feature of protein metabolism. This may involve some abnormality in the metabolism of the sulphur amino-acids since methionine is the potential precursor of choline by virtue of its ability to donate its methyl group for the biosynthesis of choline.

A study of protein metabolism in a few typical cases would show whether there is any gross abnormality in the utilization of protein or amino-acids in this disease. In addition to balance studies the pattern of urinary excretion of amino-acids, particularly the sulphur amino-acids and also of the amino-acids in the blood, should be determined. The modern technique of paper-partition chromatography and microbiological methods of assay have to be employed for the purpose.

A balance study would give the percentage utilization of fat in the cow's milk diet. A complete analysis of the fat excreted on a fat-free or very low-fat diet, e.g. skimmed milk or soya-bean milk, as compared with that on a whole-milk diet would show the nature of the excreted fat and throw light on the source of the liver-fat. The rate of absorption of the fat in cow's milk could also be determined by chylomicrographic studies. If any abnormality is thus revealed then the blood concentration of intermediary fat metabolites could be determined.

The biochemical studies suggested above would enable us to get a complete picture of the state of protein and fat metabolism in this disease and is likely to throw considerable light on its ætiology.

5. Inquiry on typhus in Bengal under Dr. K. V. Krishnan at the All-India Institute of Hygiene & Public Health, Calcutta.

Object of the work.—The object was to study the clinical epidemiological and transmission aspects of typhus fevers in general and scrub typhus in particular.

A technique for breeding *T. deliensis* in the laboratory has been developed and has been published in the *Indian Medical Gazette* (February 1949). Since presenting the last report in December 1948, the following investigations have been conducted :—

(*i*) *Clinical features of typhus.*—One hundred and sixty-seven further fever cases were investigated this year. Of these, 38 were enteric, 27 typhus, 11 kala-azar and the rest other fevers. The percentage of typhus cases among all fever cases admitted to hospital worked out to about 16 per cent. Out of the 27 cases of typhus seen, 18 belonged to OXK serological group, four to OX 19 and five gave no significant result. Due to lack of transport facilities a large number of cases could not be contacted this year.

(*ii*) *Entomological studies.*—Seven hundred and twenty-one rodents were caught for study of ectoparasites. Three hundred and four of these or about 42 per cent were found infested with mites. Shrews and *Rattus rattus* were found as before to be more often infested than the other species of rodents (shrews 55 per cent, *R. rattus* 42 per cent.)

Of the mites on the rodents *T. deliensis* was the predominating species (65 per cent) ; *Trombicula indica* and certain new species came next (30 per cent) and *S. bengalensis* came last. One of the new species of *Trombiculid* mites has been bred out in the laboratory and its identification characters worked out.

(*iii*) *Transmission experiments.*—The next important achievement this year is the successful transmission of *Rickettsia orientalis* infection to white mice through the bite of experimentally infected laboratory-bred larval mites. Out of 12 experiments done, eight were positive, thereby establishing conclusively the role of *T. deliensis* in the transmission of scrub typhus in this area. After the completion of this experiment, a paper was published in the *Indian Medical Gazette* (February 1949) on the experimental transmission of scrub typhus by *T. deliensis* to mice.

(*iv*) *Congenital transmission.*—In the transmission experiments reported above larval progeny of infected mites were used because congenital transmission occurs from parent to offspring. To find out the number of generations over which congenital transmission occurs without fresh infection certain experiments were conducted. It was found that (*i*) in 5 out of 5 experiments second generation larvæ transmitted the infection to mice, (*ii*) in 2 out of 2 experiments third generation larvæ were infective to mice and (*iii*) in one experiment using fourth generation larvæ no infection could be demonstrated as the mouse was found dead and decomposed. From the above series of experiments it looks as if, that once a mite larva gets infected, it can transmit infection to its progeny at least for three generations.

(*v*) *Trials with aureomycin.*—At the request of Lederle Laboratories, aureomycin was tried in the treatment of typhus cases. Out of the six cases of suspected on which it was tried, two later proved to be typhoid, three typhus and one, a case of cystitis following typhus. In the last four cases treatment with aureomycin brought the temperature to normal within 24 hours, and there was complete recovery in 72 hours. In the typhoid cases, the temperature was brought down a little for a few days and later the course and termination were not much affected.

(*vi*) *Cultivation of rickettsia.*—Several attempts were made to cultivate four different strains of *Rickettsia orientalis* in the yolk-sac of chick embryos. The infected yolk-sac smears were stained by the combined Leishman-Giemsa, Giemsa overnight and Machivello techniques, and examined for the presence of rickettsia. Rickettsia could not be demonstrated in any of the smears examined microscopically. On inoculation into mice, however, death was caused in about six days. The cause of death was found to be due to rickettsia as examination of peritoneal smears showed their presence. After passage through yolk-sacs rickettsia generally caused the death of mice earlier than before. Occasionally the yolk-sac material failed to cause the death of a mouse, though the material from a subsequent egg-passage proved lethal to mouse. Yolk-sac from dead embryos were also infective. One of the strains studied could be maintained in eggs up to the 10th passage in this way.

6. Inquiry on the effects of vitamins B and C in diabetes under Dr. S. Banerjee at the Presidency College, Calcutta.

The role of adrenal cortex in the altered carbohydrate metabolism in scurvy.—Adrenal cortex, which is the richest source of vitamin C and cholesterol in the body, plays an important role in the metabolism of carbohydrates. It has been observed that both the ascorbic acid and cholesterol contents of the adrenal glands are diminished after injection of the adrenotropic hormone of the pituitary gland. Injection of adrenal-cortex extract leads to the deposition of glycogen in the liver of rats, while repeated injections of such extracts lead to the development of diabetes in the animal. We have shown that the glucose tolerance, insulin content of the pancreas are greatly diminished when guinea-pigs develop vitamin-C deficiency. We have also given evidences to show that the altered carbohydrate metabolism of vitamin-C deficiency guinea-pigs is not due to the increased secretion of either thyroid hormone or adrenaline. Experiments, therefore, were undertaken to see if the adrenal-cortical hormones are involved in the altered carbohydrate metabolism in scurvy.

Four groups of guinea-pigs were uniformly selected and were fed four different diets as follows for 21 days :—

Group I ..	Scorbutic diet only.
Group II ..	Scorbutic diet with a daily intraperitoneal injection of 0·2 c.c. of adrenal-cortical extract per animal.
Group III ..	Scorbutic diet with a daily oral supplement of 5 mg. of vitamin C per animal.
Group IV ..	Same diet as in group III with injection of adrenal-cortical extract as in animals of group II.

Animals of group I were fed *ad libitum* while the corresponding animals of the remaining groups were fed the diet actually consumed by the animals of the group I. The weight of the animals were taken on alternative days.

The glucose-tolerance test was performed in all the animals on the 21st day of the experiment. The animals were killed after the glucose-tolerance test on the same day and the glycogen content of the liver and the total cholesterol content of the adrenal glands were estimated. The results are shown in Tables I to III :—

TABLE I.

Glucose-tolerance test in guinea-pigs on different diets.

Guinea-pigs on :	Fasting blood-sugar.	Blood-sugar after feeding glucose : mg. per cent.			
		45 min.	90 min.	135 min.	180 min.
Scorbutic diet only (10) ..	137	258	306	240	207
Scorbutic diet with injection adrenal-cortical extract (10)	137	237	256	249	209
Scorbutic diet with vitamin C (10)	130	217	240	191	141
Scorbutic diet with vitamin C and with injection of adrenal-cortical extract (10) ..	133	238	263	220	174

TABLE II.

Average value of the glycogen content of the liver of guinea-pigs.

Scorbutic animals, g. per cent.	Scorbutic animals with adrenal-cortical extract, g. per cent.	Scorbutic animals receiving vitamin C, g. per cent.	Scorbutic animals receiving vitamin C and injection of adrenal-cortical extract, g. per cent.
1·003	1·75	1·80	1·89

TABLE III.

Average value of the total cholesterol content of the adrenals.

Scorbutic animals mg./100 g.	Scorbutic animals injected with adrenal-cortical extract, mg./100 g.	Scorbutic animals receiving vitamin C, mg./100 g.	Scorbutic animals receiving vitamin C and injection of adrenal-cortical extract, mg./100 g.
1,467	1,360	1,854	1,990

DISCUSSION.

Table I.—The fasting blood-sugar values in all the groups did not vary. The blood-sugar values of the scorbutic guinea-pigs 45 and 90 minutes after they were fed glucose were considerably higher than the animals of the other three groups. The glucose-tolerance curve of the scorbutic guinea-pigs with or without the injections of adrenal-cortical extracts did not differ indicating thereby that the altered glucose tolerance in scorbutic guinea-pigs is not due to the diminished secretion of adrenal-cortical hormone. The injection of adrenal-cortical extract in guinea-pigs on normal diets (i.e. scorbutic diet with supplements of vitamin C) definitely lowered the glucose tolerance of the animals supporting the observation of others of the diabetogenic action of the adrenal-cortical extracts in rats.

Table II.—The injection of adrenal-cortical hormone in guinea-pigs fed a scorbutic diet only definitely increased the liver-glycogen value of the liver of these animals. Similar injections in animals on normal diet had practically no effect.

Table III.—The cholesterol content of the adrenal gland was considerably diminished in vitamin-C-deficient guinea-pigs. The injection of adrenal-cortical extract in these vitamin-C-deficient animals did not alter the cholesterol content. The lowered cholesterol content of the adrenal glands of the scorbutic animals might be due to the secretion of the adrenotropic hormone of the anterior lobe of the pituitary gland.

The animals which were on scorbutic diet only when received injections of adrenal-cortical extract the rate of the fall of their body-weights were considerably lower than that of the animals which received no injections. A steroid hormone has been isolated from the adrenal cortex which contain ascorbic acid. The supply of the hormone by injection might spare adrenal-cortical hormone and this might have helped in the diminished fall in weight of the animal on scorbutic diet.

Vitamin C and diabetes.—Studies on the urinary excretion of vitamin C in normal and diabetic persons after test doses of vitamin C and the effect of vitamin C on the glucose-tolerance test and on the urinary excretion of sugar by diabetic patients were continued and the results similar to those as reported last year were obtained.

Studies on the effect of removal of adrenal glands in normal and scorbutic guinea-pigs on the glucose-tolerance test, on the glycogen value of the liver are in progress and the work is expected to be completed by the end of the financial year.

7. Inquiry into erythroblastosis fœtalis and the Rh factor under Lieut.-Colonel S. D. S. Greval at the School of Tropical Medicine, Calcutta.

1. Seventy-five subjects were tested for iso-immunization, among them anti-body was found only in five :—

(*a*) Three of them were due to Rh.

(*b*) One of them (group O) possessing the α and β in high titre (up to 1 in 500).

(*c*) One was due to some other antigen.

2. Forty-eight cases were tested with Rh typing sera (anti-Rh anti-Rh^1 and anti-Rh^2) routinely in determining the iso-immunization with the following results :—

Rh (serologically negative)	.. 4
Rh^1 (clinically negative)	.. 1
Rh^2 (clinically negative)	.. Not found.

3. Experiments are in progress with a view to improving the anti-Rh animal serum. Considerable time has been spent on this important item which appears to be ignored elsewhere at present.

4. Iso-hæmagglutinogen content of Indian saliva has been determined. Saliva is employed in neutralizing the unwanted iso-hæmagglutinins in the serum of Rh-iso-immunized subjects. The content is poor. A paper entitled ' Low Iso (hæmagglutino) gen Content of Saliva in Calcutta ' is in press and will appear in the January 1950 issue of the *Indian Journal of Medical Research*.

5. Imported anti-Rh sera have given results which admit of doubts on the standardization of the sera and their reliability in medico-legal matters.

8. Inquiry to investigate allergic diseases and significance of endogenous allergens as regards ætiology, diagnosis and treatment of allergic diseases under Dr. J. C. Ray at the Indian Institute for Medical Research, Calcutta.

Previous note on the subject seems to have revealed that urinary proteose has some therapeutic effect as hypo-sensitizing agent in suitable cases. Its effect may be due to its antigenic nature, as antibodies have been demonstrated in patients and have been actively produced in laboratory animals. Specificity of anti-proteose-antibodies has been observed ; and if bacterial or any other microbial metabolism has anything to do with the production of urinary proteose, a secondary endogenous allergen, it may be expected that these microbial agents or their metabolites directly influence the specificity of the urinary proteose. This piece of work was planned to scrutinize and detect any such role of the microbial agent in shaping the chemical and immunologic character of the urinary proteose.

I. Identification of the bacterial entity of the urinary proteose.

Identification of the bacterial entity of the urinary proteose in allergic cases has been planned on the basis of Landsteiner's views that proteins lend to their respective antibodies absolute specificity. These specificities of proteins depend on the 'determinant' or 'hapten'—linkages specific to them, so that they may be effectively identified in terms of their antibodies. If, in the case of the urinary proteose, bacteria have at all any function to determine its specificity, it is probably by such haptenization of urinary proteose by bacterial haptens or antigens. Anti-proteose-antibodies may, therefore, be expected to exhibit such a relation with bacterial antigens, which are supposed to impart the specificity.

In this piece of investigation anti-proteose-serums were produced in the laboratory animals, rabbits and guinea-pigs. These anti-serums were cross-matched with different bacterial antigens by complement-fixation reactions. Modification of the technique has often been tried with a view to improve the efficiency of the tests. In the first part of the investigation, bacterial emulsions were used as antigens but as bacterial proteins are better antigens than emulsions or lysates, in the latter part of the work, bacterial proteins were used instead of emulsions. The reactions observed in the series being weak, the technique was further modified and tests were carried out at lower temperatures.

Experimental details.

Production of anti-proteose serum in rabbits.—Animals were injected proteose emulsions as in the previous note. Doses were 0·5 c.c., 1 c.c., 1·5 c.c., and 2 c.c., intravenously at week's intervals. The animals were bled seven days after the last injection and serums were inactivated for complement-fixation reaction. The tests were carried out on the next day.

Animals tolerated allergic proteose well and did not exhibit any untoward symptoms ; but with tubercular proteose, rabbits presented no difficulties, while to guinea-pigs such tubercular proteose seemed to be highly toxic, animals having died within one or two days even after the first injection. Post-mortem of such animals did not reveal any gross pathology, nor culture of blood did yield any pathogenic growth. Tissue-sections are being studied with the help of pathology department of a local hospital.

Preparation of bacterial antigens.—In the first portion of the work bacterial emulsions, in the strength of 100 million per c.c., were employed as antigens for cross-reactions with anti-proteose-serum, but in the latter part, bacterial proteins were employed as antigens. The method of extraction of bacterial proteins was based on Price-method (modified). To 100 c.c. of sterile normal saline, containing 200 million organisms per c.c., added 1 c.c. of N/1 NaOH. The whole mass of liquid was then put on a water-bath at 37°C. for two hours To this lysed bacterial emulsion was then added 10 per cent trichloro-acetic acid till fine flocculations occurred (usually 2 c.c. to 2·5 c.c.). The flask was then put on the bath at 37°C. for half an hour. The protein-fiocccules were then centrifuged and the precipitate dissolved in saline, 4 c.c. per 100 c.c. of original emulsion. N/10 NaOH was then added drop by drop till the precipitate went to colloidal solution. One c.c. of one per cent formol-saline was then added as preservative. The final pH of the antigen-solution was adjusted between 7·6 to 7·8.

Anti-complementary properties and antigenic qualities of these bacterial protein-antigens were tested, before employing them as antigens in tests proper with anti-proteose-serum. Anti-complementary dilutions of these protein-antigens were : *streptococcus* 1 : 4 in 1·5 m.h.d., *micrococcus catarrhalis* 1 : 4 in 1·5 m.h.d., the *pneumococcus* 1 : 4 in 1 m.h.d. and Friedlander's *pneumo bacillus* 1 : 4 in 1·5 m.h.d., the complement titre being 1 : 40. 1 : 20 dilutions of these antigens were capable of combining with respective antibodies. None of these showed any hæmolytic tendencies. Working dilutions of these antigens were : *streptococcus* 1 : 10, *micrococcus* 1 : 10, *pneumococcus* 1 : 5 and *pneumo bacillus* 1 : 5. Old tuberculin was used to serve the purpose of tuberculo-antigens in dilution of 1 : 50, as it contains the tubercular hapten.

Main test.—The technical details of the tests proper were same as given in the previous note on the demonstration of antibodies against urinary proteose. The working dilution of serum was 1 : 5 and complement was used in 2 m.h.d. The control tubes for serums and antigens contained 1 m.h.d. complement and the hæmolytic control tubes for antigen contained three volumes of antigen with no complement. The set was incubated at 37°C. for one hour and then at 22°C. for 15 minutes ; hæmolytic system was then added in each tube and readings were taken after the control tubes showed complete hæmolysis. The results of the tests may be evidenced from the Tables I and II :—

TABLE I.

Complement-fixation reaction with bacterial emulsion and tuberculin (T50) as antigens.

Case number.	Serum number.	Complement-fixation reactions with antigens of :						
		Strepto-coccus.	*Micrococcus catarrhalis.*	*Pneumo-coccus.*	*Friedlander's pneumo bacillus.*	*B. coli.*	*Salmonilla (stock-strain).*	*T 50.*
Nuro-dermatitis, P 7*	3 R	+++	—	—	—	—	—	—
	N (normal)	—	—	—	—	—	—	—
Pulmonary tuberculosis, P 11	9 G.P.	—	—	—	—	—	—	+
	N (normal)	—	—	—	—	—	—	—
Psoriasis, P 23	10 G.P.	—	—	—	—	—	—	—
	N (normal)	—	—	—	—	—	—	—
Pleurisy, P 24	11 R	—	*Trace*	—	—	—	—	++
	N (normal)	—	—	—	—	—	—	—
Intestinal tuberculosis, P 25	13 R	—	—	—	—	—	—	+
	N (normal)	—	—	—	—	—	—	—
Pulmonary tuberculosis, P 26	15 R	—	*Trace*	—	—	—	—	+
	N (normal)	—	—	—	—	—	—	—
Psoriasis (S.T.M.), P 27	16 R	+	—	—	—	—	—	+
	N (normal)	—	—	—	—	—	—	—
Rheumatoid arthritis, P 28	17 R	—	+	—	—	*	*	*
	N (normal)	—	—	—	—	*	*	*
Rheumatism, P 34	20 R	*Trace*	—	—	—	*	*	—
Adenitis, P 38	21 R	—	*Trace*	—	—	*	*	—
	N1 (normal)	—	—	—	—	*	*	—
	N2 (normal)	—	—	—	—	*	*	—

+ indicates 'inhibition' and — 'no inhibition.'

* 'P' stands for 'proteose', 'P 7' means 'proteose No. 7'.

TABLE II.

Complement-fixation reaction with bacterial protein and tuberculin (T50) as antigen.

Proteose number.	Anti-serum number.	Results of complement-fixation reaction with antigens of :				
		Streptococcus.	*Micrococcus catarrhalis.*	*Pneumo-coccus.*	*Friedlander's pneumo bacillus.*	*T 50.*
P 34 ..	20 R	+	—	—	—	—
P 38 ..	21 R	—	+	—	—	—
P 39 ..	26 R	—	*Trace*	—	—	—
*P 40 ..	27 R	—	±	—	—	++
P 42 ..	28 R	—	+	—	—	—
†TP 1 ..	30 R 31 G.P. (died)	—	—	—	—	+++
†TP 2 ..	32 R 33 G.P. (died)	—	—	—	—	+++
†TP 3	34 R 38 G.P. (died)	—	—	—	—	++
†TP 4 ..	36 R 39 G.P.	— —	— —	— —	— —	++ —
†TP 7 ..	37 R 40 G.P. (died)	+	++	—	—	++++
†TP 8 ..	38 R 41 G.P. (died)	+	—	—	—	+++
†TP 9 ..	39 R	+	++	—	—	++++

+ indicates 'inhibition' and — indicates 'no inhibition'.

* Patient had pleurisy sometime back. † Tubercular proteose.

Complement-fixation reaction at lower temperatures.—The results observed, though revealing a number of positive findings, were not very clear-cut, probably due to weak antigen-antibody reaction *in vitro*. It was, therefore, tried to modify the technique of the test in such a way as to suit most efficiently the delicacy of the reactions. Complement-fixation reaction at lower temperature, as outlined by Russian workers for testing the typhus sera, was given a trial with some modifications of the original technique.

Working dilution of complement.—Guinea-pig's serum was the source of complement. Different dilutions of complement, i.e. 1 in 10, 1 in 15, 1 in 20, 1 in 25, 1 in 30, 1 in 40, 1 in 50 and 1 in 60, were made with normal saline. 0·2 c.c. of each dilution was titrated with 0·4 c.c. each of normal saline and hæmolytic system containing three per cent suspension of sheep's r.b.c. and 5 m.h.d. anti-sheep amboceptor. Readings were taken after incubation at 37°C. for half an hour. Working dilution of complement was fixed at the dilution in the second tube from that indicating the titre of complement, i.e. if the titre was 1 : 25, the working dilution of complement was 1 : 15.

Determination of complement unit.—Complement in working dilution was then titrated with reagents serum and antigens; seven tubes for each reagent were taken. Complement, in that dilution, was then added in quantities 0·06 c.c., 0·07 c.c., 0·08 c.c., 0·09 c.c., 0·1 c.c., 0·15 c.c. and 0·2 c.c. of normal saline was added in each tube. After addition of 0·2 c.c. of reagents, serum or antigen, the tubes were incubated at 37°C. for one hour, 0·2 c.c. of hæmolytic system was then added to each tube and reading was taken after 45 minutes' incubation at 37°C. Each reagent was titrated separately for its respective complement unit. *Complement unit (c.u.) for a particular reagent was taken to be the lowest quantity of complement-dilution producing complete hæmolysis of* 0·2 *c.c. of hæmolytic system after* 45 *minutes' incubation, and the complement unit for the main test (with both serum and antigens) was the lowest common quantity of complement giving complete hæmolysis of the given quantity of hæmolytic system when titrated with reagents separately*; taking for instance, if the complement unit for *streptococcal* antigen was 0·09 c.c. and that for the anti-proteose serum 0·06 c.c., the complement unit for the main test with *streptococcal* antigen and anti-proteose serum was 0·09 c.c. Higher multiples of the unit, such as 1·5 complement unit, 2·0 complement unit, 2·5 complement unit, etc., could be accordingly calculated.

Main test.—In the main test, 0·2 c.c. serum and 0·2 c.c. antigens were taken in small sugar tubes, to them were added different complement units, usually 1 c.u., 2 c.u. and 2·5 c.u. 0·2 c.c. normal saline was used to replace serum in antigen control tubes and antigens in serum-control tubes. At the utmost, therefore, 16 tubes were scheduled for each antigen, four for serum control, four for antigen control, four for anti-serum with the antigen and four for normal serum with the antigen. The whole set of tubes was then placed inside a refrigerator (temperature 0°C. to 4°C.) for 18 hours. After the cold incubation, 0·2 c.c. of hæmolytic system was added to each tube and the tubes incubated at 37°C. The readings were taken as soon as the controls showed complete hæmolysis. Bacterial proteins were used as antigens and the working dilutions were as in the tests at 37°C. (*vide supra*). The results are given in Table III :—

Table III.

Complement-fixation reactions at lower temperature with bacterial antigens.

Proteose number.	Serum number.	Complement used, c.u.	Results of complement-fixation reaction with bacterial antigens :			
			Streptococcus.	*Micrococcus catarrhalis.*	*Pneumococcus.*	*Friedlander's pneumo bacillus.*
P 34 ..	20 R	2	+	+	—	—
		2·5	+	—	—	—
P 38 ..	21 R	2	—	+	—	—
		2·5	—	*Trace*	—	—
P 39 ..	26 R	2	—	+	—	—
P 40 ..	27 R	2	+	—	*Trace*	—
	27 R	2	+	—	+	—

c. u.=Complement-unit, + indicates 'inhibition' and — indicates 'no inhibition'.

Interpretation of results.—Results in the Tables have been indicated as in previous note by '+' and '—' signs, '+' indicating positive and '—' negative reactions. Different grediants of positive reactions have been represented by varying numbers of + signs, complete inhibition of hæmolysis by ++++ and definite inhibition by + sign.

Control.—Normal animal sera of same species as the experimental served as negative serum controls, while non-tubercular proteose samples as negative antigen control.

Scrutiny of the foregoing Tables will reveal that anti-proteose serum in rabbits gave cross-fixation with bacterial antigens, and the specificity of urinary proteose, according to the workers, is due to haptenization of proteose by bacterial antigens. Such an allergen in tubercular cases may be the missing link in the observation by Sigurdsson of 'masked antigen' in tubercular tissues (*Jour. Immunol.*, **51**, p. 279, 1945).

II. DEMONSTRATIONS OF ANTI-PROTEOSE ANTIBODIES IN HUMAN SERA.

In view of fundamental scientific and diagnostic importance, this problem has, at present, been confined to tubercular case only. Primarily antigens for such tests are being prepared from such tubercular proteose-samples which contain only the tubercular and not other bacterial 'haptens', i.e. TP 1, TP 2, TP 3, and TP 4 and not TP 7, TP 8 and TP 9 (*cf.* Table II). As such, 'contamination' with other heterogenous, non-specific bacterial 'hapten' may have a possibility of giving false positive reactions amongst the control series. Aim of the workers is to embrace as much 'polyvalency' as possible by including different samples from different cases in one combined poly-antigen for the test. Technique of antigen-antibody reaction is by the process of complement-fixation and details are as reported earlier. Frank tubercular cases are being tested with such poly-antigens against the negative antigen-control of non-tubercular proteose and negative serum-control from tuberculin-positive and tuberculin-negative healthy individuals. The help of the B.C.G. team at Calcutta is being sought for the purpose.

9. Inquiry on the immunological properties of lens and uveal proteins under Dr. S. N. Cooper at the Seth G. S. Medical College, Bombay.

The work hitherto carried out under the inquiry can be grouped under the following heads :—

(A) Preparation of material for clinical and experimental trials.

(B) Chemical analysis of the samples for testing.

(C) Preparation of a lens anti-toxin as a standard for comparing the antigenic property of the different lens preparation.

(D) Animal experiments.

(E) Clinical trials.

A. PREPARATION OF MATERIAL.

The following lens preparations have been made :—

(1) Saline extract of bovine lenses. (2) Ammonium hydroxide extract of bovine lenses. (3) Extract of bovine lenses in distilled water. (4) Suspension in distilled water of human lens cortex from the following five kinds of lenses : (*a*) Normal lenses, (*b*) immature cataractous, (*c*) ripe cataractous, (*d*) hypermature Morgagnian, and (*e*) black cataractous. (5) Dried extract of ripe cataractous lenses as prepared in distilled water is described separately in *Appendix A.*

An explanation is required for the preparation of so many varieties of the extracts.

1. Saline extract gives the maximum amount of lens extract by way of total solids.
2. An ammonium hydroxide extract gives the maximum yield of crystalline α of lens protein.
3. A distilled-water extract is needed for comparison.
4. The extracts of different types of human cataractous lenses are needed to determine the difference in the antigenic value of the different types of cataractous lenses, which may give a clue to the activating factor in the antigenic property of lens proteins which may be responsible for iritis phaco-anaphylactica and sympathetic ophthalmia. For the present a suspension is made in distilled water only (*see* below).

We are trying to measure the antigenic property of lens protein by testing them against a standard lens anti-serum, which we have prepared and stocked, by determining the quantity of precipitins. So far we have measured them only in Widal tubes but we are going to measure the same more accurately by means of turbidity filters in a photo-colorimeter, which is due to arrive from America. This will give us a clue which solvant gives the greatest yield of antibody-producing lens proteins. We shall add that solvant to the distilled-water suspension or their dried extracts of the different types of cataractous lenses to produce the maximum yield of soluble lens proteins peculiar to each and evaluate the antigenic property of each variety of human cataractous lenses.

5. This method of getting lens protein in a dry form (described in the *Appendix A*) has not hitherto been made elsewhere as far as we know. It will do away with the problem of losing some of the protein during the filtration of the lens suspension through a bacteriological filter.

It will give the soluble lens substance in a dried form which can be preserved indefinitely and from which any required concentration of the solution can be made.

B. CHEMICAL ANALYSIS.

Chemical analysis of the different types of lens extracts will be made.

From an ammonium-hydroxide extract of bovine lenses the α and β fractions have been isolated, in a relatively pure form by the method stated in *Appendix B*.

The Officer-in-Charge of the inquiry is going to take advantage of his visit abroad to study the electro-phoretic pattern of the molecules of the different types of lens extracts.

C. PREPARATION OF A LENS ANTI-TOXIN.

By injecting rabbits with a progressively increasing dose of lens protein we have succeeded in producing a fair amount of lens anti-serum, a 1/1,000 dilution of which goes with a one per cent solution of ammonium-hydroxide extract.

Our first rabbit, after the injectable dose had been raised to 10 c.c., got anaphylactic shock and died. Subsequently we have kept three animals for each experiment.

Anti-serums against the saline lens extract, ammonium-hydroxide extract, and distilled-water extract, are ready.

Denoting AS as anti-serum and AG as lens antigen the following matchings will be made :—

Distilled water AS + distilled water AG.
,, ,, AS + ammonium-hydroxide AG.
,, ,, AS + saline AG.
Ammonium-hydroxide AS + ammonium-hydroxide AG.
,, ,, AS + saline AG.
Saline AS + saline AG.

This will give us an idea for the most suitable solvant required for experimental and clinical trials.

D. ANIMAL EXPERIMENTS.

Animal experiments are given in the attached scheme.

E. CLINICAL TRIALS.

Clinical trials are being continued with whatever samples of lens extract available and we continue to get gratifying results in post-operative inflammations.

A critical study of the cases has been started with the ammonium-hydroxide lens extract standardized against the stock anti-serum for standardizing.

APPENDIX A.

Method of preparation of the lens extracts in dried form.

The method is known as freezing method.

In the case of bovine eyes the lenses are taken out from the eyeballs by a transverse corneal incision and then by cutting the capsule.

In the case of human cataractous lenses, the lenses that are extracted intra-capsularly are taken, washed lightly in normal saline and collected in a sterile bottle containing 30 c.c. sterile distilled water, 10 drops of 5 per cent carbolic acid and sterile broken glass. Ten lenses are taken for every 30 c.c. of distilled water. The different bottles contain different types of cataractous lenses. The bottles are then shaken for 18 hours in a shaking machine and kept for a week in a freezing chamber of a refrigerator after the lenses appear properly macerated.

Then this suspension of lens matter is thoroughly centrifuged thrice till the supernatant fluid appears slightly opaque.

Twenty c.c. of the supernatant fluid is measured and put in a glass-ampoule and kept in a cooling chamber at —30°C. till it is hard frozen (in about two hours).

The ampoule is then connected with an exhaust pump and kept immersed in methyl acetone in a beaker with a vacuum of 200 μ (0·2 mm.).

After the extract is dried the ampoule is sealed in vacuum.

The advantage of this technique are :—

1. In this state the extract can be kept indefinitely without fear of decomposition.

2. There is no chance of losing the proteins which are lost during the passage of the lens extract through a bacteriological filter for sterilizing.

3. We can prepare the lens extract of any strength wanted for our work by dissolving the same in the required amount of sterile distilled water.

4. Moreover, there is no chance of getting the lens extract contaminated by any means.

5. No antigenic property will be lost.

However, it is not correct to say that the whole substance is a pure lens-protein moiety.

Animal experimentation scheme.

Group O.	Group A.	Group B.	Group C.
Three rabbits	Three rabbits	Three rabbits (in each sub-group)	Three rabbits
	Sensitize to *strepto-staphylo* toxin in the following doses, and then traumatize lens :— 0·5 c.c. 1·0 c.c. 1·5 c.c. 2·0 c.c. 3·0 c.c. 4·0 c.c. 5·0 c.c. (at five days' interval) (intravenous)	Sensitize with whole lens protein selecting from the preparation that gives the greatest yields of antibody forming protein (*see* under C) 0·5 c.c. 1·0 c.c. 1·5 c.c. 2·0 c.c. 3·0 c.c. 4·0 c.c. 5·0 c.c. Group B$_1$ (0·5–1·0 c.c.) Group B$_2$ (0·5–3·0 c.c.) Group B$_3$ (0·5–5·0 c.c.) (at 5 days interval)	Sensitize with mixture of toxins and lens.
	Rest for 10 days. Test serum for specific antibodies.	Rest for 10 days. Test serum for specific antibodies.	Rest for 10 days. Test serum for antibodies against lens and toxins separately.
Traumatize lens if no reaction in the eye sensitize to *staphylo* toxin and use it for control in the second half of experiment in group B.	Traumatize lens.	Traumatize lens. If no reaction in the eye then sensitize to *strepto-staphylo* toxins using GO as control.	Traumatize lens. If no reaction rabbits unsuitable for these experiments or *strepto-staphylo* toxoid unsuitable for stepping up antigenic property of lens protein. To use a different sensitizing agent tubercle bacilli.

To remove the eye after inflammation and examine histologically particularly to exclude changes due to mechanical factor giving rise to vascularization and hyperæmia only due to trauma and hypotony and take culture from iris of enucleated eyes to exclude infection.

APPENDIX B.

Separation of ∝ and β crystallin from bovine lens emulsion where different solvants, namely, N/100 ammonium hydrate solution, normal sodi-chloride and distilled water have been used.

Twenty bovine lenses, taken out after transverse corneal incision were collected in a sterile serum bottle containing broken glasses and 200 c.c. of any of the above solvants, measuring 10 c.c. for each lens with 20 drops of carbolic acid, one drop for each lens.

The bottle was kept for a day in a ice chamber and the lenses thoroughly macerated for 24 hours in a shaking machine and then kept in an ice chamber for 48 hours without disturbing the fluid.

The solution was then centrifugated twice at 2,500 revolution per minute and the thinner supernatant fluid was separated and collected in a separate flask.

Separation of ∝ and β fractions.

0·8 c.c. of approximate N/10 glacial acetic acid was added per each 10 c.c. of the thinner supernatant fluid to make the solution up to the pH 4·8 (this should be done in separate tubes and measured amount of acid should be added). A heavy precipitate settled down. The same fluid was kept in an ice chamber for *two nights and days* without any disturbance.

If the supernatant fluid after keeping in the ice chamber for two nights and days, comes to be water clear, the clear supernatant fluid contains β-crystallin. If it becomes hazy, some ∝-crystallin is within the β-crystallin solution.

Again add the approximate N/10 glacial acetic acid in a measured amount, so that the hazy supernatant fluid comes to be water clear.

The supernatant fluid should be syphoned off in a separate flask from the ∝-precipitate which should be dissolved by N/100 ammonium hydrate, and this treatment should be repeated twice.

The supernatant fluid obtained by the second operation should be thrown off.

The precipitate and the supernatant fluid (in the 1st operation) thus obtained are ∝- and β-crystallin respectively in a pure form.

The ∝-precipitate should be dissolved by N/100 NH_4OH using approximately per gm. precipitate 10 c.c. of NH_4OH N/100.

For sterilization the Seitz filter is the best of all filters, as lesser amount of protein is lost when the solution is passed through the Seitz. It has been found that when the solution is passed through the B. filter for sterilization, the total solid content of the filtrate is lesser than that obtained from the Seitz filter (*see* Table). Before passing through Seitz filter, N/10 (approx.) sodi-bicarbonate should be added to make both the solution of ∝ and β crystallin up to the pH of 7·4 for intravenous injection.

When sodium chloride (normal) solvant is used for dissolving bovine lenses, the β-crystallin obtained by the above way is found to be 0·7 per cent total protein in the filtrate obtained through Seitz filter.

After making the solution sterile the solution is put in an empty sterile penicillin bottle. The contents of each bottle after filling with the sterile extract should be tested for sterility.

When the solution is sterile, the bottles are stocked in a ice chamber.

The protein estimation should be done from the contents of each sterile bottle.

The sterile extract should be injected intravenously in the ear vein of rabbits in an increasing doses (according to the contents of the proteins) on two or three days' interval to produce specific antibodies. The serum obtained after giving increasing doses of ∝-crystallin does not show any precipitin reaction when titrated with β-crystallin antigen ; but it shows precipitin reaction +++ in 1 : 1,000 dilution when titrated with ∝-crystallin. The anti-serum is preserved as standard anti-serum.

10. Inquiry on the neurogenic factor in the production of traumatic shock and its treatment by alteration of cerebro-spinal electrolytes under Dr. P. K. Sen at the Seth G. S. Medical College, Bombay.

The work was simultaneously conducted on two separate lines :—

1. Experimental work on dogs.
2. Treatment of series of clinical cases of shock.

The solution recommended by Dr. Lena Stern of II Moscow Medical Institute was used for both experimental and clinical purposes, e.g. 1/6 gramme molecular solution of mono- and di-hydrogen potassium phosphate, with pH adjustment at 7·6, sterilized and ampouled in neutral glass-ampoules. The sterility of the solution was confirmed bacteriologically.

1. Experiments on dogs.

Dogs were anæsthetized (general), mainly by urethane, intramuscularly ; sometimes intratracheal ether and chloral hydrate were tried but urethane was found most satisfactory for the purpose. The carotid blood-pressure was recorded directly by means of a canula in the neck connected through a mercury manometer to a recording revolving drum. The respiratory movements were recorded directly through a tracheotomy tube connected sideway to a recording tambour. Cisternal puncture was done before shocking began, shock being produced by different agents and methods. Blood-pressure was allowed to drop to at least half the original level before the solution was injected. The amount of solution injected varied from 0·5 c.c. to 10 c.c., either given in single or multiple repeated doses.

Various methods were used to shock the dog, e.g. :—

1. Repeated slow bleeding.
2. Intramuscular and intravenous injection of sodium nitrite.
3. Intramuscular and intravenous injection of histamine phosphate.
4. Intestinal strangulation.
5. Fracture of both femurs.
6. Deep general anæsthesia (urethane).
7. Injection of toxic substances : (*a*) Novocaine 1 per cent intravenous, (*b*) percaine, (*c*) potassium cyanide.
8. Injection of peptone.

The following were noted :—

1. It was noticed that there was an immediate (within ½ minute) rise of blood-pressure after the injection in all types of shock.

2. The magnitude of the rise of blood-pressure depended on :—

(*a*) The amount of potassium-phosphate solution injected within limits.

(*b*) The degree to which the animal was shocked. The response suffered both in magnitude and duration as the level of blood-pressure at the time of injection became less and less till at last there was very feeble and short-lived response and at last there was no response at all. As much as 0·25 c.c. of solution produced a marked pressor effect in animals which were not shocked, while 5 c.c. to 6 c.c. produced no response or feeble response in markedly shocked animals.

3. The duration of the response depended on certain factors, e.g. :—

(*a*) The degree to which the blood-pressure had fallen at the time of injection. It became less and less as the fall was more.

(*b*) Whether the injection was first or repeated ; the second response was less in duration and magnitude when given under similar circumstances, e.g. at the same level of blood-pressure.

The average duration, i.e. the time taken to rise and fall to the level at which the injection was given, was about 15 minutes. The maximum was 25 minutes, while the minimum was only one minute.

The duration of the response was tried to be enhanced by giving the injection by the drip method intracisternally. Even under these circumstances the duration was not more than 25 minutes. These experiments again confirmed that :—

(*a*) The response became less and less as the injection continued.

(*b*) That there was some deleterious effect on the respiration.

4. The potassium ion seems to have deleterious effect on the respiratory centre at least in dogs. It was noticed that the constant cause of death was respiratory arrest and failure. The respiration failed although the blood-pressure was high or normal, sometimes 5 to 10 minutes after the injection. The level of blood-pressure could be maintained and animal kept alive if the artificial respiration was started immediately. A second injection of potassium phosphate under these circumstances produced an immediate pressor-response but failed to re-start the respiration, suggesting the presence of functioning vasomotor centre but failed respiratory centre. The possibility of the medullary damage by the needle, especially in view of shallow cisternal magna, was borne in mind and due care was taken in performing the cisternal puncture and while injecting the solution. It was also noticed that there was no response to the injection of solution, in the form of rise of blood-pressure, when the medulla was damaged intentionally with the needle. The possibility of respiratory arrest due to alteration of pH of the CSF does not arise as the pH of the solution is the same as that of the CSF, e.g. 7·6.

5. In the toxic types of shock produced by novocaine, percaine and KCN, the response was not marked.

6. It was not possible to produce anaphylactic shock in dog by means of peptone injection as the dog does not seem to be susceptible to it.

It seems from the above observations that the seat of action is the stimulation of the medullary and hypothalmic sympathetic centres, and depends upon the presence of intact and functioning of these centres *plus* the functioning peripheral arterioles. The toxic shock is refractory to the treatment because the drugs like novocaine, percaine and KCN depress the very centres which we seek to stimulate by the intracisternal injection of potassium phosphate.

7. A few control experiments were performed. Solution of normal saline was injected intracisternally, both slowly and w
of Hg) when the solution was injected with force (mechanical stimulation).

In conclusion the pressor-response of intracisternal injection of potassium phosphate in shocked animals is a definite and c
The response, however, varies both in duration and degree.

In all 41 animals were experimented upon. The results of the experiments are given in Table I.

TABLE I

CHART SHOWING THE RESULTS OF INTRACISTERNAL INJECTION OF POT

Dog.		Pre-	Anaesthe-	Shocking	Initial B.P.	B.P. just before	Maintenance Time	Total Amount of K	Character and time of	Time taken to rise to	Level of peak	Time taken to fall to	Level of subsequent fall	Average level of mainten-	Time of mainten-
31	8·875kgs.	..	,,	2. gr. Na No 2 IV	95 ,,	42 ,,	3 mts.	7·5 cc	,,	3 mts.	78 ,,	28 mts.	52 ,,	70 ,,	25 mts.
32	7·25kgs.	..	,,	Peptone, and 2 gr. Na No 2 IV	114 ,,	50 ,,	5 mts.	2 cc	,,	4 mts.	134 ,,	15 mts.	60 ,,	1·5 ,,	12 mts.
33	5·875kgs.	..	,,	Peptone and 2 gr. Na No 2 IV	110 ,,	58 ,,	5 mts.	2 cc	,,	1 mt.	202 ,,	7 mts.	128 ,,	180 ,,	5 mts
34	9·25kgs.	..	,,	Peptone and 6 mg. Histamine 1, intraperitonially	110 ,,	50 ,,	5 mts.	2 cc	,,	1 mt.	68 ,,	19 mts.	40 ,,	50 ,,	15 mts.
35	10·625kgs.	..	,,	Repeated 0·1 gr. KCN IV Total 1·1 gr.	140 ,,	. ..	..	..	Dog	died sudde	nly after	2 hrs.	2 hrs.		7 mts.
36	7·5kgs.	..	,,	Repeated KCN Total 1·6 gr.	118 ,,	20 ,,	Suddenly Resp. stopp	4 cc ed	..	..			..		
37	9·125kgs.	..	,,	% Novocine IV Repeated Total 7 c.c.	132 ,,	72 ,,	2 mts.	1 cc (Resptopped)	,,	2 mts.	88 ,,	8 mts.	66 ,,	70 ,,	5 mts.
38	8·5kgs.	..	..	1% Percine Repeated Total 9 c.c.	100 ,,		Dog died su	ddenly	ıd R.S. a	nd C.S.V.	failure			..	..
39	6·125kgs.	..	..	% Percine Repeated 0·5 c.c. Total 3·5 c.c.	108..	←——	Suddenly r	espn.	coinues. In	j. 3 c.c. K.	effect on	B.P.			
40	6·875kgs.	..	..	0·5 % Percine 5 c.c. Repeated Total 1 c.c.	88..	25 ..	Suddenly Respn. stop	2 cc ped—2	.. ċ. artifl.	respiration	————	————	————	————→	
41	5·625kgs.	..	,,	0·5% Percine 5 c.c. Repeated Total 1 c.c.	30 ,,	10 ..	..	2 cc	..	1·5 mts.	30 ,,	16 mts.	18 ..	20 ..	15 mts.

easily secured. Majority of cases was
impossible. In others blood-pressure

variable. As opposed to experiments
Certain side effects, such as vomiting,
y and 2 c.c. as the usual dose, but

OM TRAUMATIC SHOCK

Results		
ne- (on te k)	Remote (ultimate result).	Remarks.
-	Recovery.	No fluids were necessary. Patient was treated conservatively. No operation necessary. Recovered.
	Death.	No fluid were necessary before operation. Patient recovered from shock, 6 days later abdominal swelling and pain developed. Operated. Rupture spleen and pancreatic necrosis ; Splenectomy. Death after 2 days.
l	do.	Intravenous saline was needed. Patient operated upon. Died next day.
	do.	Patient died half an hour later.
	do.	Saline intravenously was needed. Operated mesenteric. tear. Internal hæmorrhage. Died ½ hour after operation.
	do.	Intravenous saline started. Patient died after 2 hours.
	do.	Intravenous fluids started. Patient died after 3 hours.
	Recovery.	Operated upon. No fluids necessary before operation. Tear in mesentery perfora-

11. Investigation into the chromatophorotropic hormone of the pituitary gland and its employment in the treatment of pigmentary disorders of the skin under Dr. B. Mukerji at the Central Drugs Laboratory, Calcutta.

Object of the work.—Investigation on the chromatophorotropic (melanophore pigment stimulating) hormone of the pituitary gland with particular reference to its therapeutic application in pigmentary disorders of the skin in tropics.

A. *Purification and concentration of the hormone.*—In continuation of our last year's work vigorous efforts were made to prepare a potent melanophore preparations. It was felt that on this depended the solution of the problem and its possible application in the treatment of human skin conditions. The problem was attacked from two different angles :—

(*i*) To make the hormone biologically free from other pituitary hormones.

(*ii*) To concentrate the hormone as far as is possible.

These have entailed a large amount of spade work, as isolation of a hormone of this type is acknowledged on all hands to be a most difficult procedure. Some success can be claimed during this year and it is hoped that the results gained so far will pave the way for the evaluation of successful method for the preparation of the hormone in a much concentrated form and freed practically from most of the other hormones present in the anterior and posterior portion of the pituitary gland, which, as is well known, is a regular storehouse of hormones of very similar but slightly different make-up.

The influence of different solvants upon extraction of the melanophore hormone from ground acetone-dried whole pituitary was studied extensively. Extraction with 2 per cent $Ca(OH)_2$ solution, 2 per cent $Ba(OH)_2$ solution, aqueous ammonia, aqueous pyridine, 2 per cent saline, 2 per cent acetic acid solution were studied. From the activity of the extracts and solid matter present, acetic acid appeared to be the solvant of choice. Extraction with $Ca(OH)_2$ seemed more potent in activity than that with acetic acid, the value being in the ratio of 48·5 : 34·5, but the ratio of the solid matter in the two extracts being 0·0143: 0·0090, acetic acid seems to surpass $Ca(OH)_2$ in so far as the activity per mg. of the extracted solids is concerned. Alkaline extraction further possessed the disadvantage that a large number of other pituitary hormones was simultaneously extracted, thus making subsequent purification more complex and cumbersome. Acid extraction in comparison circumvents this difficulty to a considerable extent.

The removal of inert matter present in the extract (made with acetic acid) with $Ba(OH)_2$, trichlor-acetic acid, lead acetate, Kieselguhr were tried. $Ba(OH)_2$ seemed to bring down very little impurities, the amount of solid matter decreasing only from 0·0090 g. per c.c. to 0·0070 g. per c.c. Trichlor-acetic acid also brought down a very small amount of solids. The supernatant liquid remained turbid and made subsequent operation difficult. Lead acetate could bring about a concentration of 1·5 times in the hormone preparation, but certain hormone was lost in the process, probably by absorption on the surface of the precipitate. In comparison, Kieselguhr furnished the most useful tool. It brought down the solid matter from 0·0095 g. per c.c. to 0·00465 g. per c.c. without causing any loss in the activity of the hormone. Certain adulterant hormones also seemed to be partially absorbed and their nature and concentration are at present under investigation.

From the Kieselguhr-treated extract the hormone was completely precipitated with four volumes of acetone at low temperature and the precipitate when dissolved in the initial volume of acetic acid, yielded a solid matter of 0·0033 g. per c.c., showing a further stage of concentration of the hormone. The precipitate, however, could be dissolved in a much less volume of very dilute acetic acid, thus effecting a concentration of about seven times at this single stage.

Changing of pH of the solution to about 9·0 at this stage by adding 10 per cent ammonia and keeping overnight brought down a further amount of inert matter without any significant loss in hormone activity and from the ammoniacal solution the hormone could be quantitatively absorbed on active charcoal. Elution of the dried charcoal with liquid phenol at different temperature was tried. Elution at 50°C. (for about an hour with liquid phenol) seemed better. From the phenol eluent the hormone could be precipitated with acetone. Unfortunately, however, this process entailed a great loss in the activity of the hormone, though biological purification was thus very successfully attained. The possibility of increasing the former, without any loss in the latter objective, is now under scrutiny. The biological test for the presence of other hormones in the acetone precipitate under question is now being carried out.

The melanophore hormone possesses the property of partly passing out in the dialysate, when a solution containing the hormone, is dialysed in a cellophane bag against distilled water (Abramowitz *et al.*, *Jour. Biol. Chem.*, **151**, p. 579, 1943). Basing on this property attempts were also made to free it from other pituitary hormones, adreno-corticotropin was a possible hormone, adulterant, its property being similar (Tyslowitz, *Science*, **98**, p. 225, 1943) and attempts are being made to measure its concentration, and to free the melanophore hormone by taking help of its solubility in 60 per cent alcohol (in which the later hormone is insoluble) by its elution with water from chemical adsorbate and by such other properties.

Another method under trial is the chloroform-extraction procedure of Schwenk *et al.* (*Jour. Biol. Chem.*, **147**, p. 535, 1943). Acetone-dried whole pituitary-gland powder was extracted at 2°C. to 3°C. with a definite volume of water at pH 8 to 9 for about four hours, at the end of which the pH was adjusted near neutrality and about twice its volume of water and an equal volume of chloroform were added and stirring was continued for two hours more and then centrifuged in a cold room within 5°C. Three layers were formed. The lower chloroform layer contained the lipid matter ; the middle 'gel' layer contained some anterior pituitary hormones, but no melanophore hormone, the latter being concentrated in the first aqueous layer along with the posterior hormones and some other anterior pituitary hormones, e.g. gonadotropin, thyrotropin. The possibility of separating the melanophore hormone by electro-dialysis (adreno-corticotropin being now absent, as it passes to the 'gel' layer) or by absorption on charcoal and elution with suitable solvants under most favourable conditions are at present being worked out.

Paper sent for publication : " Studies on the Chromatophorotropic Hormone of the Pituitary. Part I ".

B. *Effect of drugs upon melanin dispersion of fish.*—In the past, the activity of melanophore-hormone preparation has been tested by injecting the active preparation into normal light-adapted frogs. An intense blackness of the skin occurs in such animals as a result of dispersion of the melanin granules of the dermal melanophores. The specificity of this test has later on been, however, questioned because a large number of other substances especially drugs have been reported to give this same reaction.

The first extensive investigation was made by Fuchs (*Biol. Centralbl.*, **26**, pp. 863 and 888, 1906) who found darkening of the skin with coniine, nicotine, veratrin, brucine and morphine. Others who participated in similar work included Hoghben and Winton, and Houssay and Ungar, as well as a host of other workers. Drugs tested also were different and included acetyl-choline, atrophine, pilocarpine, caffeine, chloral-hydrate, chloretone, chloralose, urethane, phentothal-sodium, barbital-sodium, coramine, etc. The results arrived at are often conflicting and contradictory to those found by others, probably because of different species of frogs used as well as difference in the environmental conditions. Teague *et al.* (*Jour. Pharmacol. & Exper. Therap.*, **65**, p. 115, 1939) reviewed the whole problem and studied the effect of drugs on both normal and hypo-physectomized frogs (*Rana pipiens*). They found that, while darkening of the skin of normal frogs was produced with a certain number of drugs, e.g. pilocarpine-HCl, chloretone, chloralose, urethane, pentothal-Na, brucine, etc. in the case of hypo-physectomized frogs, no effect was produced except with posterior pituitary extracts and hence claimed that the hypo-physectomized frogs (*Rana pipiens*) was a very specific test object for melanophore hormone, ' proved that a very dark gross appearance of the skin with complete expansion of melanophores to the reticular phase is obtained before referring the results as positive test '.

As regards the question as to why the drugs which acted on the normal frogs failed to elicit any response in the hypo-physectomized ones, they could not furnish any definite explanation, but suggested that the drugs might act on the *pars intermedia* directly or indirectly through the nerves (he himself expressed doubt on the latter).

That blackening of the skin of fish like ' Magur ' (*Glarius bratracus*) results when the melanophore preparation is injected into such fish, initially made pale by keeping in a white background and exposed to light, was reported last year. To find out whether drugs had got any part to play in the migration of the melanin granules of their skin, a large number of drugs, e.g. urea, histamine-phosphate, ephedrine-HCl, morphine-sulphate, digitaline, urethane, chloral-hydrate, coramine, gardenal-sodium, ergometine, atropine-sulphate, pilocarpine-nitrate, pentothal-sodium, chloralose, brucine, chlorobutol, phenoburbitol-sodium, etc., were injected into such fish and their effects observed. It was found that except posterior pituitary extract, pitressin (P. & D.) pentothal-sodium and chloralose which produced darkening within one hour, no other drug caused any significant migration of the melanin granules. Several other drugs remain to be tested as yet ; but from the results so far gained, it can be concluded that the ' Magur ' fish as such cannot act as a specific test object for the melanophore hormone.

Incidentally, the results also invalidates the suggestion of Teague *et al.* (*loc. cit.*) that drugs act directly on the *pars intermedia ;* for it cannot explain as to why the drugs, e.g. brucine, chloretone, etc. which produced blackening by acting on frog pituitary, failed to elicit any response by similarly acting on the pituitary of ' Magur ' fish. Further work on this problem is in progress.

Paper ready for publication : ' The Effect of Drugs upon the Melanin Dispersion of Fish-skin '.

C. *The influence of melanophore hormone upon the melanin contents of fish-skin.*—A large number of ' Magur ' fish (*Glarius bratracus*) was divided into four groups as shown below :—

Group A : Those which were kept in white background.

Group B : Those which were kept as those in group A, but injected twice daily for three consecutive days each time with 0·1 c.c. of our potent melanophore preparation (1 mg./c.c.).

Group C : Those which were kept in black background.

Group D : Those which were kept as those in group C but were injected with melanophore preparation exactly as those in group B.

At the end of three days all the fish in four groups were killed with chloroform vapour and the skin of each group of fish were pooled together and assayed for the melanin content in accordance with the method of Summer *et al.* (*Biol. Bull.*, **84**, p. 187, 1943) a photo-electric colorimeter being used for the final estimation of melanin.

It was found that, while the melanin content of skin of fish kept in black background (group C) was significantly greater than that of fish kept in white background (group A), no significant differences were noticed between the melanin contents of groups A and B fish or between those of groups C and D. Thus, the melanophore hormone may not have any direct influence on the absolute increase of pigment but only brings about a migration of pigment cells. Further work on this problem is in progress.

12. Inquiry into the state of refraction of Indian children of different communities with special reference to myopia under Dr. G. S. Pendse at the Talegaon General Hospital, Poona.

The basic standards of the state of refraction of Indian children of different communities have not been worked out. It is intended to work out the refractive state of some homogeneous communities, such as the Parsees and Brahmins. Simultaneously, the cases which can be classified as pathological myopes, will be taken up for detailed investigation as regards their heredity and constitution particularly with a view to study the constitutional factor in myopia.

Plan of work and expectations from the collections of data.—The first part of the work, viz. the standards of the state of refraction, will be worked out by doing a series of atropine refractions in children of two age groups (6 to 12 and 12 to 18) and the curves will be drawn to study the incidence of emmetropia, hypermetropia and myopia. The second part of the work, viz. the investigation of the pathological myopes, from the point of view as to the constitutional element as a factor in its ætiology and progress, has been started. Data are being gathered regarding their heredity, height, weight, diet and the blood picture which includes hæmoglobin, differential count, serum calcium and plasma-proteins. The cases are chosen particularly below the age of 20, the age during which this disease usually progresses, since the refraction tends to become stationary at and round about the age of 20. It is interesting to note that in about 25 cases gathered so far, there is either a deficient serum-calcium or a high lymphocytic count. It appears that there is some factor in the constitution which helps the progress of the disease during the period of the body-growth, irrespective of the factor of heredity. The collection of data in this direction is likely to throw some light on this factor. During the collection of the basic data of refraction, it is also intended to collect the data with regard to heights, weights, hæmoglobins, differential counts, of the same age groups and the same communities to compare them with the findings of the myopes, both pathological and physiological. We have already started the work of taking the normals of the hæmoglobins and differential counts in the age groups mentioned above in the community which we are taking for the study of the refractive state.

13. Study of *Monilia* infection of the skin and adjacent mucous membrane under Dr. L. M. Ghosh at the School of Tropical Medicine, Calcutta.

During this period 75 cases have been studied. Clinical study of the cases examined so far reveal the following result :—

Total number of cases examined ..	75
Monilia isolated	38

This is divided into five groups as follows :—

Group I .. *Perionychia.*—This is the commonest variety of infection encountered so far. History of long duration with occasional improvement was usually obtained from the patient. Painful œdematous condition of the surrounding tissue with disfigurement of the nails have been found in all. All the cases in this group were females.

Cases examined	20
Monilia isolated	13

Group II .. *Lesions in the mouth.*—In this group the clinical characteristic is whitish patches (*i*) on the mucous surface of the lip, especially the lower lip, (*ii*) at the corners of the mouth with cracks at the muco-cutaneous junction (may or may not be accompanied with a nutritional deficiency) and (*iii*) on the dorsum of the tongue.

In some cases the patients complained of burning sensation during meals, in others there were no complaints excepting the appearance of the patches.

	Number of cases examined	27
	Monilia isolated	11
Of which:—	Number of cases with lip involvement	12
	Monilia isolated	4
	Number of cases of glossitis	7
	Monilia isolated	4
	Cases with lesions at the corners of the mouth	8
	Monilia isolated	3

Group III .. *Lesions in the webs of the fingers and toes.*—Whitish sodden patches with cracks were seen in the webs between the fingers of the hands and toes of the feet. Ringworm infection of the adjacent areas were eliminated. In some of these cases the lesions were ulcerated.

Cases examined	14
Monilia isolated	6

Group IV .. *Vulvo-vaginitis.*—One case was examined and found positive.

Group V .. *Tropical eosinophilia of the lungs.*—

Material examined (sputum)	17
Monilia isolated	6

Group VI .. *From an ulcer on the forearm.*—*Monilia* was isolated in one case.

METHOD OF INVESTIGATION.

A. *Identification of the species.*

Total number of *monilia* isolated	38
Species identified so far (the remaining strains are being studied)	30

Following species have been identified :—

Monilia pinoyi	15
M. nabarroi	7
M. krusei	3
M. bronchiatis	3
M. balcanica	2
Total	30

B. *Agglutination reaction.*

In detection of serum antibodies for *Monilia*, agglutination test has not been used widely and there is no standard technique available. Much difficulty has been experienced at first regarding the method of the experiment.

The following technique was followed :—

The serum was first inactivated at 56°C. for half an hour. It was then diluted with normal saline to 1/10, 1/20, 1/50, 1/100, 1/200, 1/500, 1/1,000. The antigen used was the culture of *Monilia*, 24-hour growth in solid Sabouraud's medium diluted approximately to 1/10th to match with a standard opacity (artificial standard). The agglutination experiment was put up with 0·25 c.c. of serum and 0·25 c.c. of antigen, antigen control was kept to check the result. The whole thing was then put in the water bath at 56°C. for 1½ hour and then kept in the ice-chest overnight. The reading was taken on the following morning (method of Norris and Rawson).

Observations.—

(*a*) Agglutination with the normal human serum takes place up to 1/50 dilution. In two cases the agglutination reached up to 1/100.

(*b*) Agglutination with the serum of positive cases takes place up to 1/500 dilution of serum. This was the maximum obtained. Majority of cases gave agglutination reaction up to 1/200.

(*c*) The results were not always uniform and further experiments are to be done to corroborate the findings and improve the method of agglutination.

C. *Animal experiment.*

The animal experimental work has not yet much advanced. Saline emulsions of live-cultures have been injected intraperitoneally to the rabbits starting from 1 c.c. and gradually increasing the dose to immunize the animal against the particular species. After seven injections (i.e. 1 c.c., 1·5 c.c., 2 c.c., 2·5 c.c., 3 c.c., 3·5 c.c. and 4 c.c.) blood was taken from the heart and the agglutination reaction was tested against the specific species and also against another species. In specific case the agglutination reaction was positive up to 1/200 and in other up to 1/50 dilution of the serum.

14. Inquiry on the problems of typing typhoid bacilli by means of Vi-bacteriophage and treatment of typhoid fever with type-specific Vi-'phage under Drs. R. G. Dhayagude and D. D. Banker at the Seth G. S. Medical College, Bombay.

Five hundred typhoid cultures isolated and made available by various bacteriological laboratories in Bombay city have been tested against the 24 Vi-bacteriophage preparations supplied by Dr. Felix. The cultures have been supplied by the Central Clinical Laboratory, K.E.M. Hospital, Haffkine Institute, Pathology Laboratory of the J. J. Hospital, Pathology Laboratory of Nair Hospital, Dr. Gajjar's Laboratory and several other individual pathologists. The collection, therefore, is fairly representative of all localities and sections of population in the city.

The Vi-'phage-typing scheme of Craigie and Felix for typhoid bacilli is applicable to local strains. The 500 typhoid cultures studied so far fall into 11 of the 24 types described by them in 1947. The types met with are :—

Vi-'phage type.		Number of cultures.	
A	..	249	
B_2	..	2	
D_1	..	9	
D_6	..	2	
E_1	..	64	
G	..	1	
J	..	2	
K	..	21	
M	..	1	
O	..	36	
T	..	3	
'Imperfect' reactions		110	(including those confirmed by Dr. Felix.)
Total	..	500	

The commonest type in Bombay is type A which is met with in about 50 per cent of the cultures tested. The other common types are E_1, K and O. It is proposed in future to study the possibility of differentiating type A cultures by biochemical reactions.

It will thus be seen that about 20 per cent of the cultures give reactions that are not true to any known type, even though they do contain the Vi-antigen. Such cultures are provisionally labelled 'imperfect', and are being studied further. It is possible that some of these might belong to types hitherto unknown. Of the 15 such strains forwarded to Dr. Felix for opinion in February 1949, three were reported by him to belong to known types, one as doubtful K type, six as degraded Vi-strains, and five as untypable strains. In this connection he also mentions that during the past few months four new Vi-'phage types of *S. typhi* have been identified by workers in different parts of the world. These new types and 'phages will probably be made available to us shortly, when the local strains giving 'imperfect' reactions can be checked up against these. The cultures from Bombay reported by him as untypable were tested by him with the four new 'phages but still remained untypable. It is intended in near future to forward to him the other 'imperfect' strains for opinion. The rather high proportion of 'imperfect' strains is due to many cultures being old and degraded. Among the freshly isolated cultures the proportion of 'imperfect' strains is much less, i.e. about 10 per cent of the cultures tested.

Among the cultures that have been tested there were a few supplied by the Haffkine Institute which were isolated in the year 1941-42 and kept in sealed tubes of nutrient agar with a little broth added. Many of these showed presence of sufficient Vi-antigen to enable typing.

The availability of specific Vi-'phages prompted us to try if it could be conveniently applied to treatment of patients whose organisms typed. Of 20 such patients 10 were given the specific Vi-'phage intravenously 1 c.c. diluted in 500 c.c. glucose saline by slow-drip method. A majority of these were, as would be expected, type A cases, about which there are doubts whether it is strictly a specific type. In these the therapy had to be repeated. During repeat therapy the powerful non-specific 'O' 'phage that we have was mixed to see if the effect was enhanced. Spectacular effect with resolution of temperature by lysis was found in only three cases. Three cases died.

The reactions with intravenous 'phage therapy were found to be too severe for general application. In 7 cases, therefore, the type-specific 'phage (mixed with 'O' 'phage when the type was A) was given intramuscularly in a dose of 5 c.c. at a time at repeated intervals. Different dosage schedules were tried varying from once a day to every three hours for two days. This mode of therapy with typhoid 'phage has not been tried generally due to fear of producing local abscesses. In our series there was no local reaction in any case except pain of injection, but apparently in most cases there was insufficient distribution of the 'phage into general circulation as rigor following the injection occurred only in two cases and spectacular effect in *nil*. It is probable that our indifferent results as compared to those of Knouf *et al.* may be due to many of the cases belonging to type A or because no special methods were employed for purification or concentration of the 'phage. It is concluded that the complicated method of preparation of type-specific 'phage, the attendant severe reactions, and the indefinite results render the therapy impracticable for general use, specially when easily administrable promising new anti-biotics have been discovered.

Incidentally, four cases were treated with aureomycin and six with chloromycetin, mostly because they did not type or did not react to type-specific 'phage.

Result.

Treatment.	Number.	Types.	Cured.	Died.	Spectacular benefit.
Type-specific Vi-'phage, intravenously ..	10	8A 2K	7	3	3
Mixed Vi + O-'phage ..	6	A	6	..	1
'Phage, intramuscularly ..	7	4A 2E_1 1 ?	7	..	..
Aureomycin ..	4	3A 1 ?	4	..	..
Chloromycetin ..	6	3A 2 ? 1E_1	3	3	3

During the year, 75 *S. paratyphi* A cultures have been tested in Bombay, most of them locally isolated and made available by the laboratories listed above. These were put up against the two specific 'phages known, 46 of the cultures fell into one or the other type but 29 cultures gave irregular reactions, which are being further studied. These strains will shortly be forwarded to Dr. Felix for further investigation. No obvious new type has been detected among these 75 cultures besides the two types already identified. Our preliminary attempts with *S. paratyphi* A have been mentioned by Dr. Felix on page 285 of the *Proceedings of the Fourth International Congress on Tropical Medicine and Malaria*, Washington, 1948. It is felt that a fairly large number of paratyphoid A cultures will have to be studied before a typing scheme similar to that existing for either typhoid or paratyphoid B bacilli can be built up. For this purpose requests have been sent round to various bacteriological laboratories all over India to preserve and collect the cultures that may be isolated, so that more paratyphoid A cultures from different regions be made available for study.

Incidentally, a semi-permanent collection of typhoid and paratyphoid A cultures is being made on lemco-agar stabs, including all the cultures that have been tested in Bombay, in case they may serve useful in future to any worker.

15. Inquiry on circulatory changes in severe infections under Dr. R. N. Chaudhuri at the School of Tropical Medicine, Calcutta.

It has long been recognized that peripheral circulatory failure is an important complication of all severe infections. The mechanism responsible for the circulatory changes is obscure, but there is evidence to suggest that peripheral collapse is accompanied by alterations of hæmodyamics ; with these considerations in mind, this inquiry was started in April 1949 to investigate the changes in plasma-volume, extra-cellular fluid (available thiocyanate space) and total circulatory proteins along with other relevant factors.

The work was begun by studies on the methods of investigation, but some difficulties were encountered at first to standardize the techniques. The method of Cooper and Morris (1944) using alcoholic phospho-tungstic acid to precipitate the plasma-proteins was found unsuitable due to evaporation of alcohol during centrifugalization and was subsequently replaced by the method of simultaneous determination of plasma-volume and available thiocyanate space (Gregersen *et. al.*, *Amer. Jour. Physiol.*, **125**, pp. 142-152, 1939). After preliminary observations on monkeys the technique was applied to the human beings. The thiocyanate-space determination was, however, handicapped owing to febrile reactions following the injections. This was eventually overcome by sterilization of the sodium-thiocyanate solution by Seitz filter and subsequent sterility tests. The work so far carried out relates to studies on 25 normal individuals, 7 cholera cases, 13 enteric, 2 typhus, 6 malaria and a few with other diseases.

METHOD.

The subjects were selected from hospital ward patients. The test was done before the principal morning meal in case of healthy persons but with no special reference to feeds in acute cases. The height and weight were recorded at the same time in cases where possible, and during the convalescent period in some. The body-surface in square metre was calculated from weight and height using DuBois line chart.

The syringes, needles and test-tubes were cleaned, dried and sterilized by dry heat. After a veni-puncture without stasis a control sample of blood (8 c.c.) was withdrawn with 20-gauze needle. The syringe was then disconnected leaving the needle in place and the blood put in an oxalated bottle. Through the same needle, 10 c.c. of five per cent sodium thiocyanate solution using a different syringe were slowly injected. Immediately after the injection the syringe was rinsed twice by drawing and re-injecting blood and then disconnected from the needle. Through the same needle, 5 c.c. of 0·7 per cent sterile solution of T-1824 dye (Evans blue) were injected from a calibrated syringe which was also similarly rinsed. After an interval of 20 minutes a veni-puncture was performed in the opposite arm, and a dyed specimen of blood was drawn and put in another oxalated bottle. Both the samples of blood, dyed and undyed, were centrifuged and the plasma separated, the concentration of the dye being determined in a colorimeter. To 1 c.c. of the dyed sample, 1 c.c. of 20 per cent trichloracetic acid was added to precipitate the proteins along with the dye ; 1 c.c. of the clear supernatant fluid was then withdrawn and treated with Reissner's reagent (ferric-nitrate solution) and the thiocyanate content is determined in the colorimeter with the help of calibration curve. Readings were first taken in a Pulfrich photometer and subsequently in an Evelyn photo-electric colorimeter. In the control samples of plasma, known amounts of suitable dilutions of the dye and the thiocyanate solutions were added and these were passed through the same processes as the unknown samples and the readings were compared. Blood-volume was determined from the plasma-volume by using the relation :—

Blood-volume $= \frac{\text{Plasma-volume}}{100 \text{ hæmatocrit}} \times 100$. From the control samples plasma-proteins were also determined by the copper-sulphate specific-gravity method, albumin and globulin by the Biuret method, plasma-chloride by modified Whitehorn's method, hæmatocrit by Wintrobe's method and hæmoglobin by Hellige method.

STUDIES IN NORMAL PERSONS.

A useful check on the accuracy of our methods has been a study of healthy normal people. So far, 25 adults (male) have been studied with the following results :—

		Range.		Mean.
Plasma-volume (c.c.)	per kg. ..	36·09 —	61·64	46·55
	per sq. m. ..	1,372 —	2,135	1,632
Blood-volume (c.c.)	per kg. ..	65·91 —	106·5	82·97
	per sq. m. ..	2,453 —	3,880	2,872

The mean average figures are in close agreement with those reported by other workers : (Gibson and Evans, 1939 : plasma-volume 41·5 c.c./kg., blood-volume 77·7 c.c./kg. ; N. C. Dutt, 1948 : Plasma-volume 48·0 c.c./kg. blood-volume 82·9 c.c./kg.).

Studies on the estimation of available thiocyanate space in normal individuals are in progress, and in a few cases where it has been estimated, the findings conform with the results of the other observers, and this was about 25 to 30 per cent of the body-weight (250 to 300 c.c./kg.).

FINDINGS IN ACUTE INFECTIONS.

I. *Enteric fever.*—Thirteen cases were studied of which three died and in six cases repeated examinations were made. The results of 13 cases on admission were as follows :—

		Range.	Mean.
Plasma-volume (c.c.)	per kg. ..	31·51 — 74·40	50·17
	per sq. m. ..	1,039 — 2,090	1,576
Blood-volume (c.c.)	per kg. ..	53·32 — 113·4	78·2
	per sq. m. ..	1,731 — 3,029	2,484

Figures per kg. weight appear to be slightly raised when compared with those per sq. m., and is probably due to disproportionate loss of weight in this disease. There seems to be some increase of the plasma-volume in average cases but it is distinctly much below the normal in three fatal cases. Thus, there appears to be two distinct groups of cases with different results. More observations will be necessary to differentiate them clearly, as this has practical bearing on treatment.

Available thiocyanate space.—This was estimated in seven cases including one fatal case. The results were as follows :—

	Range.	Mean.
Per kg. ..	400·8 — 234·9	300·30
Per sq. m. ..	11,250 — 5,000	8,872

Total circulating plasma-proteins.—Estimation of plasma-proteins indicates, as before, a lowering in its concentration, the mean value for these 13 cases being only 5·7 g. per cent, much lower than our normal values of 7·1 g. per cent. While expressing this result in terms of total circulating proteins in the blood per kg. body-weight (estimated from the plasma-volume) the average value is 2·8 g./kg. as against 3·3 g./kg. in the normal. This lowering is evident, in spite of the fact that the mean value for the plasma-volume is higher in these cases than the normal. This is a significant lowering of the mean value but here also one group of cases shows much more reduction due to contracted plasma-volume, while the other group show little or no change due to increased plasma-volume. The amount of total circulating proteins is important because this indicates the amount of oncotic pressure which blood can exert in holding its fluid content. Any marked reduction of oncotic pressure will adversely affect its capacity in performing this function with the resultant reduction in the plasma-volume.

Plasma chloride.—This, however, shows no significant variation from the normal although the urinary chloride excretion remained definitely low (about 0·2 g. per cent average) throughout the period of illness. This shows that the kidney excretes chloride prudently to the maximum economy of the body, without much disturbance of blood-chloride so as to maintain the electrolytic environment of the body cells within optimum level.

Follow up.—In six cases where plasma-volume was again estimated during convalescence, the results were as follows :—

		On admission.	During convalescence.
Plasma-volume (c.c.)	per kg. ..	49·28	51·5
	per sq. m.	1,555	1,614
Blood-volume (c.c.)	per kg. ..	71·2	79·74
	per sq. m.	2,466	2,561

The difference is of not much significance and no conclusion can be drawn unless a large number of cases is examined especially after complete recovery of the previous health of the individual.

Of the three fatal cases, in two where clinically peripheral failure was detected, the plasma-volume and also the total circulating proteins were alarmingly low and in the third case which died of encephalitis, the result was not far from the normal as shown below :—

Cases.	Circulating plasma-proteins g./kg.	Plasma-volume (c.c.)		Blood-volume (c.c.)		Blood-pressure (mm. Hg.)
		Per kg.	Per sq. m.	Per kg.	Per sq. m.	
1.	1·98	32·0	1,039	52·32	1,731	60/45
2.	2·2	37·45	1,400	58·55	2,152	90/45
3.	3·03	72·6	2,047	113·4	3,026	120/70

II. Other cases.

(1) *Typhus :* In two cases of scrub typhus, plasma-volume was estimated on four occasions during fever and the result was 47·17 c.c./kg. and 1,526 c.c./sq. m., blood-volume was 69·69 c.c./kg. and 2,259 c.c./sq. m. Total circulating plasma-proteins was 2·8 g./kg.

(2) *Malaria :* In six cases of malaria, during febrile period the results obtained were as follows :—

		Range (c.c.)			Mean (c.c.)
Plasma-volume	per kg. ..	50·4	—	70·2	62·6
	per sq. m. ..	1,600	—	2,133	1,912
Blood-volume	per kg. ..	90·0	—	106·7	98·2
	per sq. m. ..	2,845	—	3,231	3,034
Available thiocyanate space (5 cases)	per kg. ..	175·4	—	318·4	247·2
	per sq. m. ..	5,710	—	9,154	7,787
Total circulating proteins (gm./kg.)	..	2·5	—	3·3	2·75

Here also both plasma-volume and blood-volume are above the normal figure and the total circulating proteins is diminished.

(3) *Cholera :* In seven cases of cholera, plasma-volume was determined immediately after admission before any parenteral fluid was administered. Although the clinical conditions, especially the state of hydration, were dissimilar in these cases the mean values obtained as noted below are significant :—

		Range (c.c.)		Mean (c.c.)
Plasma-volume	per kg. ..	27·47	47·18	40·04
	per sq. m.	1,013	1,579	1,353
Blood-volume	per kg. ..	67·48	97·30	84·05
	per sq. m.	2,360	3,410	2,780

The plasma-volume was very much contracted in three cases, but compensatory polycythæmia with increased hæmatocrit value (range—42 to 64 per cent, mean 51·1 per cent) does not reflect much alteration in the values for blood-volume. In two cases, plasma-volume was estimated on a subsequent day, after recovery and the readings tended to return to normal.

(4) *Miscellaneous :* Various estimations have also been done in some other cases, viz. splenomegaly, anæmia, epidemic dropsy, etc.

Further work is in progress.

16. Hæmatological unit under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta.

I. ANÆMIA IN PREGNANCY.

Sixty-one pregnant women were studied at the ante-natal clinic of the Eden Hospital. These cases could be classified in the following way :—

	Cases.
(*a*) Macrocytic anæmia	22
(*b*) Hypochromic anæmia	5
(*c*) Dimorphic anæmia	20
(*d*) Normocytic anæmia	14

Many of these patients are receiving appropriate hæmatinic treatment and are being followed as far as possible.

II. ROLE OF VITAMIN C IN THE UTILIZATION OF IRON FOR ERYTHROPOIESIS.

Anæmia patients with hypochromic or dimorphic blood picture and cellular normoblastic marrow are selected for this purpose. Seven patients were studied during this period :—

(*a*) Four patients were given orally small doses of ferrous sulphate (gr. 6 daily) with 100 mg. of vitamin C for a period varying from three to five weeks. The optimum improvement, which is usually found after gr. 18 daily dose of ferrous sulphate, was noted with this small dose given along with vitamin C in two cases. In the other two cases, the improvement was also fairly satisfactory though later on further improvement of blood picture was noted with full dosage of ferrous sulphate (gr. 18 daily).

(*b*) Gr. 12 ferrous sulphate given along with 100 mg. of vitamin C for 30 days effected optimum erythropoiesis in one case.

(*c*) Gr. 12 ferrous sulphate with 100 mg. of vitamin C given for 20 days effected good erythropoiesis in a patient who had shown only slight improvement with gr. 18 ferrous sulphate given previously for 18 days.

(*d*) In one case only moderate improvement was effected with gr. 12 ferrous sulphate given along with 100 mg. of vitamin C. Later, further improvement was noted after the administration of full dose of ferrous sulphate (gr. 18 daily).

III. SERUM IRON LEVEL IN CASES OF ANÆMIA.

The level of serum iron is being determined according to Powell's modification of Heilmeyer's method, in the different types of anæmias.

In cases of hypochromic anæmia, serum-iron is being determined in the anæmic state before iron therapy is instituted, during the course of treatment with full dose of ferrous sulphate as also with small dose of iron given along with 100 mg. to 200 mg. of vitamin C, and finally after the course of iron treatment. The work is being continued.

IV. ASSAY OF FOLIC ACID.

(*i*) *Assay of free folic acid.*—The basal medium for microbiological assay has been standardized. Test assay with free folic acid has given highly satisfactory results. This unit is now in a position to estimate the free folic-acid content in samples of liver extract.

(*ii*) *Assay of folic-acid conjugate.*—The assay of folic-acid conjugate is being carried out according to the method suggested by O. D. Bird *et al.* This work is still in progress.

V. HÆMATOLOGICAL STUDIES IN BUBONIC PLAGUE.

Hæmatological investigations and Paul Bunnel test were carried out on 46 cases admitted in the infectious ward of the Campbell Hospital with clinical diagnosis of suspected plague. Of these cases *Pasteurella pestis* was found only in nine cases by gland puncture. Mean hæmatological values in confirmed cases of bubonic plague were as follows :—

Hb.	R.b.c.	M.c.v.	M.c.h.	M.c.h.c.	Total w.b.c.	Neutrophils.	Lymphocyte.	Monocyte.	Eosinophil.
13·3 gs.	3·84	103·0	34·5	33·4	9,266	7,589	1,126	529	22

E.S.R. (Wintrobe, 1 hour reading, corrected for anæmia) .. 40·5 mm.
Prothrombin time (modified Quick technique) .. .. 0′-30·2″
Toxic neutrophils and Turk's irritation cells were found in four patients.

Mean hæmatological values in the remaining 37 cases in which the diagnosis was not confirmed by bacteriological examination were as follows :—

Hb.	R.b.c.	M.c.v.	M.c.h.	M.c.h.c.	Total w.b.c.	Neutrophils.	Lymphocyte.	Monocyte.	Eosinophil.	Basophil.
13·17 gs.	3·77	98·5	34·8	33·2	8,870	6,448	1,565	629	260	45

E.S.R. (Wintrobe, 1-hour reading corrected for anæmia) .. 39·0 mm.
Prothrombin time (modified Quick technique) .. .. 0′-27·8″
Toxic neutrophils were seen in three cases, neutrophilic myelocytes in two cases and Turk's cells in four cases.

Paul Bunnell test was done on 38 cases. In none of the cases could heterophil antibodies be demonstrated beyond a dilution of 1·40.

VI. URETHANE IN THE TREATMENT OF CHRONIC MYELOID LEUKÆMIA.

During the period three cases of chronic myeloid leukæmia were treated with urethane (ethyl-carbamate).

(*a*) A female patient, aged 27 years, was given 3 g. of urethane daily for 32 days. Initial white cell count of 275,000 per c.mm. came down to 7,700 with almost complete disappearance of immature cells from the peripheral blood. Along with the fall in white cell count, there was definite clinical improvement with significant rise in both r.b.c. and hæmoglobin level. Two weeks after the withdrawal of the drug there was further reduction in the white cell count which came down to 6,000 per c.mm. One week later, the blood picture again showed further rise in r.b.c. and hæmoglobin level, but contrary to expectation the white cell count went up to 52,000 per c.mm. with re-appearance of many immature cells in the peripheral circulation.

(*b*) A male patient, aged 49 years, was given urethane in dosage of 3 g. daily. The dose was later on reduced to 2 g. daily as the patient was complaining of nausea. This patient altogether received 101 g. of urethane in 46 days during which period the initial white cell count of 136,000 dropped to 33,000. Along with the fall in white cell count there was clinical improvement with slight increase of r.b.c. and hæmoglobin.

(*c*) A girl, aged 12 years, was given urethane in the dosage of 0·6 g. daily for a period of 42 days, at the end of which the white cell count fell from 49,600 to 6,800. Unlike the previous two cases, however, the general clinical condition definitely deteriorated with further reduction of hæmoglobin and r.b.c. The case could not be followed as the patient was taken out of Calcutta by her father.

17. Inquiry on the associated vaccine for prophylactic immunization against typhoid, paratyphoid, cholera, dysentery and tetanus by a single inoculation under Dr. J. C. Ray at the Indian Institute for Medical Research, Calcutta.

Since the beginning of the inquiry it has been the aim to proceed critically and to direct the work on more fundamental lines. From the enormous literature that has been accumulated, it now seems to the workers that the essential problem in the preparation of such a potent associated vaccine as under the inquiry, is a successful working out of a method for immunization against the dysentery infections. Combination of separate unrelated antigens for effecting prophylactic immunization is no longer considered beyond the realm of feasibility.

The opposition so far existing as to the use of combined antigens, brought forward by the supporters of 'the theory of antigenic competition' has been exploded and simultaneous immunization with more than one antigen has been achieved. On the basis of these observations the hypothesis of antigenic synergy has come to light. However, the varied nature of the work covering a wide range, brings us to a stage where the intensity of the work should embrace more limited problems so that a definite advance under the proper control in the field of combination of separate bacterial antigens against multiple infections may be reached. With this end in view the initial study has been limited at present to the problem of dysentery immunization.

The problem of dysentery prophylaxis is a difficult one from the standpoint of diversity of the types as well as from the standpoint of the toxicity of the antigens concerned. Only with complete and thorough knowledge of the antigenic fractions, their nature, components and commonness, the preparation of a potent antigen, comprising all antigenic fractions of the members of the dysentery bacillus, can be suitably taken up.

The relative importance of the various fractions derived from the complete antigens of Shigella bacillus in the production of various endotoxic reactions have been demonstrated. The reactions produced by the injection of complete antigens is due to the presence of conjugated protein as well as the undergraded polysaccharides while the corresponding simple compounds without the specific phosphorus group is inactive. The so-called phosphorus group attached to the carbohydrate fraction of the antigen acted either as an 'injury producing' or as a 'preparing' agent. The pathogenic activity of the complete antigen has been ascribed to the polysaccharide fraction and to a lesser extent on the conjugated proteins. The phospholipin component has been separated from the antigenic complexes by Morgan even without acid, alkali or enzymic hydrolysis. This phospholipin-free material is found to be fully antigenic to rabbits and induce Shiga hetrophile immune bodies.

The work is at present proceeding in the following lines :—

I. *Extraction of chemical antigens by various techniques.*—Isolation of antigenic constituents from Gram-negative bacillus has been reported by a number of investigators. Most notable among these are Boivin *et al.* (1935) who have studied the specific antigens of *Salmonela typhimurium*, *S. enteritidis*, *B. typhosum*, *E. coli*, and Topley *et al.* (1937) who have described the specific 'H' and 'O' antigens from enzymatic breakdown product of *S. typhosa.* Perhaps the most comprehensive study regarding the nature of antigenic fraction from these Gram-negative organisms is that of Morgan and Partridge (1937, 1938, 1940, 1942) who have studied in detail the specific antigens of Shiga and typhoid bacillus. Boivin *et al.* and Topley *et al.* regarded these substances as belonging to a class of compounds known as lipo-carbohydrates. It was, however, shown by later workers that proteins or protein-like constituents form an important part of type-specific antigens derived from Shiga and typhoid bacillus (Morgan and Partridge, 1937) and Flexner strains (Walther *et al.*, 1945).

In view of the opinion held by later workers that no single method is effective in removing all the specific antigens, attempts are being made to extract the antigens by different techniques as described in the literature, the immunological activity being made the basis of standardization of such extractions by different methods. The methods of extraction that are being followed are given below :—

(*i*) Tryptic digest method of Topley *et al.* (*Brit. Jour. Exper. Path.*, **15**, p. 113, 1934).

(*ii*) Solvant extraction method of Morgan *et al.* (*Biochem. Jour.*, **31**, pp. 940 and 2003, 1937 ; **34**, p. 169, 1940 ; **35**, p.1140, 1941).

(*iii*) Extraction with aqueous pyridine with subsequent acetone fractionation after the method of Walther (*Jour. Exper. Med.*, **81**, p. 315, 1945).

II. *Studies of the synergistic effect of combined bacterial antigens.*—

Preparation of the antigens : The bacterial antigens were prepared after the tryptic digest method of Topley and Raistrick.

Antigen lot No. I — Tryptic digest of *B. shiga.*
,, ,, ,, II — ,, ,, ,, *Flexner.*
,, ,, ,, III — ,, ,, ,, *Cholera.*
,, ,, ,, IV — ,, ,, ,, *Para A.*
,, ,, ,, V — ,, ,, ,, *Para B.*
,, ,, ,, VI — Combination of I to V in equal quantities.

Experimental animals : Young white rats varying in weight from 30 g. to 45 g. were arranged in six cages with three rats in each.

Dosage of antigen and intervals between injections : Starting with 0·25 c.c./kg. body-weight intraperitoneally increasing the subsequent doses as follows :—

2nd dose — double the 1st dose.
3rd dose — same as 2nd dose.

Injections were given every 72 hours.

Antibody response : Animals were bled five days after the 3rd injection ; antibody response were tested by agglutination reaction at 56°C. for 24 hours. The results are represented in the Table :—

TABLE.

Antigens injected.	Titre of antibodies against homologous strain:				
	Shiga.	Flexner.	Cholera.	Para A.	Para B.
Individual antigens (I-V) ..	1/500	1/500	1/200	1/500	1/500
Mixed antigen (VI) ..	1/500	1/500	1/200	1/500	1/1,000

The above experiments show the synergistic effect of a number of different antigens. Antigen VI has been prepared by a combination of equal quantities of antigens I to V, the individual constituents are, therefore, diluted five times. The antibody response in animals with antigens, five times less concentrated, is, however, practically the same, if not better, as in the case of para B.

III. *Determination of the optimum dose of complete antigens as prophylactic agent.*—The immunogenic use of bacterial products for experimental immunization of man has been reported by a number of investigators. Somatic antigens of typhoid (Morgan *et al.*, 1943) and Shiga dysentery bacillus (Morgan *et al.*, 1943) have likewise been found to evoke agglutinating and mouse-protecting antibodies in human subjects. Grasset (1939) and Boyd (1943) have shown that a marked lowering of morbidity and mortality in a large group of individuals resulted from inoculation of typhoid endotoxin. Though it would seem that circulating antibodies do not necessarily reflect the state of local tissue immunity in the gastro-intestinal tract (Besredka, 1927) and local infection of the tract may not be prevented by parenteral vaccines, the evidence gathered from carefully controlled studies seems to suggest that vaccine may have a prophylactic role in the control of intestinal dysentery (Paddle, 1938).

In all such studies objectives were to eliminate or minimize the toxic effects which accompany the use of whole vaccine, but so far as Gram-negative bacterial vaccines are concerned, such a procedure has failed to serve the purpose as toxicity of such antigen is an inherent property of somatic antigens, and de-toxication means loss of immunogenic activity. The hopeful aspect in such an investigation with such a highly toxic substance, is that such chemical antigens are more highly potent than the whole bacterial vaccines. ' Injection of 0·1 mg. subcutaneously in humans induce the formation of more mouse-protective antibody than did 2·5 c.c. of two standard bacterial vaccines and was accompanied by less severe local and constitutional reactions (Morgan *et al.*, 1943).' Attempts are being made to determine the optimum dose of these complete antigens on animals with a control series of bacterial vaccines.

IV. *Determination of the role of adjuvants in vaccines.*—Comparative study was made (Walther *et al.*, 1945) in experimental animals of antibody production and tissue changes, following the injections into the foot-pads of saline-in-Falba and mineral oil emulsions, of killed bacteria in saline, and of killed bacteria in Falba-mineral-oil emulsions. It was found that the antibody production was greatly prolonged by emulsification of bacterial bodies in oil. While with antigen in saline, the serum titres began to fall nine days after the injection and disappeared somewhere between three and six months, with antigens in paraffin oil they began to drop only after 14 days and were still high, after 10 months, when the experiment was ended. The toxic effect of the antigens were greatly reduced by emulsification in oil. Oil drops remained detectable in foot-pads until the end of the experiment. Bacteria remain visible in oil for one week or more, but in saline they disappear within one day.

Killed *Mycobacterium tuberculosis* has a further synergistic effect (Freund, 1944) in such a water-in-oil vaccine emulsion. It has been shown also that lipid of such acid fact bacteria, has the same effect as entire bacteria. In view of this fact and the fact that killed Leishmania bodies are rich in lipid content, these being far less toxic bodies are proposed to be tried as synergistic agent with the mineral oil adjuvants for the preparation of such a vaccine.

18. Inquiry on the histological examination of the brain of *Micro-chiroptera taphozous melanopogen* with special reference to auditory tracts and medial geniculate body under Dr. (Miss) L. Chacko at the Christian Medical College, Vellore.

It is known that bats depend upon their auditory system for their movements. They produce supersonic sounds inaudible to the human ear, which on reflection from obstacles indicate their position and the animal avoids them even when blind-folded. It was the object of the inquiry to study the internal ear and the auditory pathways in the brain of micro-chiroptera taphozous melanopogan (a small variety of bat).

The procedure of the study was as follow :—

1. Attempts at successfully rearing the micro-chiroptera taphozous melanopogan and to keep them alive for long periods for future experimental work were made. It was found that, in order to keep them alive under laboratory conditions, complete darkening of the room was essential.

2. Study of the external features of the brain and the internal ear were made with special reference to the auditory system. The cochlea was relatively large. The cochlear nerve was found to be the thickest of all cranial nerves. It was extremely difficult to isolate it for the purpose of preparing histological section due to the fact that the base of the cochlea is closely applied to the side of the Medulla oblongata. The trapezium, lateral fillet, inferior colliculus, inferior brachium and medial geniculate body are remarkably prominent, and their size is out of proportion to that of the neighbouring parts.

3. In order to investigate further the highly developed auditory system of the bat, various histological and staining techniques have been applied so that on the basis of a knowledge of the normal microscopic structure, degeneration experiment may be done later. The preparation of satisfactory stained section of the normal brain and cochlea has taken several months. At present the work is at this stage.

List of papers published on clinical research.

BANERJEE, S., GHOSH, N. C., and BHATTACHARJEE, G.—A chemical method of estimation of nicotinic acid in urine in the presence of sugar (*Jour. Biol. Chem.*, **172**, p. 495, 1948).

BANERJEE, S., GHOSH, N. C., and BHATTACHARJEE, G.—Studies on the effect of nicotinic acid on the blood sugar and urinary excretion of sugar of normal and diabetic rabbits (*Ind. Jour. Med. Res.*, **36**, p. 341, 1948).

BANERJEE, S., and GHOSH, N. C.—Effect of nicominide on blood sugar and blood acetone bodies of diabetic and normal subjects (*Jour. Biol. Chem.*, **177**, p. 789, 1949).

CHAUDHURI, S. N., and RAY, J. C.—Nature and specificity of urinary proteose. Part I. Clinical trials of auto-proteose in allergic cases (*Ann. Biochem. & Exper. Med.*, **9**, No. 4, 1949).

CHAUDHURI, S. N., and RAY, J. C.—Nature and specificity of urinary proteose. Part II. Demonstration of antibodies against urinary proteose, using it as an antigen (*Ann. Biochem. & Exper. Med.*, **9**, No. 4, 1949).

CHAUDHURI, S. N., and RAY, J. C.—Nature and specificity of urinary proteose. Part III. Study on the relation of urinary proteose with the bacteria suspected to be the primary factor for the condition. (*Ann. Biochem. & Exper. Med.*, **9**, No. 4, 1949).

Nature and specificity of urinary proteose. Part IV. Study of anaphylaxis in rabbit against urinary proteose (*Ann. Biochem. & Exper. Med.*).

Study on the biochemistry of allergic blood. Part I. Study on sodium potassium, chlorides and protein ratios (*Ann. Biochem. & Exper. Med.*).

DAS GUPTA, C. R., and CHATTERJI, J. B.—Magnkaryocyte in peripheral blood (*Ind. Jour. Med. Assoc.*, **18**, No. 6).

REPORT OF THE SEVENTH MEETING OF THE CLINICAL RESEARCH ADVISORY COMMITTEE

Held in the Central Secretariat, North Block, New Delhi on the 25th and 26th November, 1949 at 10.00 a.m.

MEMBERS PRESENT.

Dr. V. R. Khanolkar. (*Chairman*).
Dr. R. N. Chaudhuri.
Dr. R. N. Cooper.
Dr. C. R. Das Gupta.
Dr. M. G. Kini.
Dr. P. Kutumbiah.
Dr. B. Mukerji.
Colonel S. Narain.
Dr. J. C. Patel. (*Secretary*).

Dr. B. B. Yodh was unable to attend the meeting.

VISITORS.

Lieut.-Colonel M. L. Ahuja.
Brevet-Colonel R. N. Chopra.
Dr. S. N. Cooper.
Group-Captain D. N. Chakravarti.
Dr. P. V. Gharpure.
Dr. A. N. Goyle.
Lieut.-Colonel S. D. S. Greval.
Dr. J. C. Gupta.
Dr. S. W. Hardikar.
Lieut.-Colonel Jaswant Singh.
Major S. L. Kalra.
Dr. K. V. Krishnan.
Lieut.-Colonel C. K. Lakshmanan.
Dr. V. R. Naidu.
Lieut.-Colonel D. P. Nath.
Dr. H. Patil.
Dr. M. V. Radhakrishna Rao.
Dr. K. V. Venkatraman.

Item 1.—To review the work in progress and consider the question of continuation or otherwise of the inquiries sanctioned during the year 1949-50.

(A) The Committee recommended the continuation of the following inquiries :—

(*i*) Clinical research unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

(*ii*) Neuropathological unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

The Committee noted that the trained personnel of the unit would be transferred to the polio research unit in Bombay.

The Committee recommended that steps should be taken to fill up the post of Assistant Research Officer on the unit which had been kept in abeyance and that provision for pay and allowances for this post made in the budget.

(*iii*) Inquiry on the role of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay.

The continuation of the inquiry was recommended with a revised budget of Rs. 10,800/-.

(*iv*) Clinical and biochemical investigations on certain aspects of infantile hepatic cirrhosis under Drs. V. Subrahmanyan and V. R. Naidu at the Central Food Technological Research Institute and Sri Krishnarajendra Hospital, Mysore.

The continuation of the inquiry was recommended with a revised budget of Rs. 11,450 -. The Committee further recommended that the post of Assistant Research Officer on the inquiry should not be continued after 1950-51.

(*v*) Inquiry on typhus in Bengal under Dr. K. V. Krishnan at the All-India Institute of Hygiene & Public Health, Calcutta.

The continuation of the inquiry was recommended with the suggestion that Dr. Krishnan should pay more importance to two features of his inquiry (*i*) complement-fixation test and (*ii*) life-cycle of mites and ticks.

(*vi*) Inquiry on the immunological properties of lens and uveal proteins under Dr. S. N. Cooper at the Seth G. S. Medical College, Bombay.

(*vii*) Investigation into the chromatophorotropic hormone of the pituitary gland and its employment in the treatment of pigmentary disorders of the skin under Dr. B. Mukerji at the Central Drugs Laboratory, Calcutta.

The continuation of the inquiry was recommended with a revised budget of Rs. 17,950/-.

(*viii*) Inquiry into the state of refraction of Indian children of different communities under Dr. G. S. Pendse at the Telegaon General Hospital, Poona.

(*ix*) Study of *monilia* infection of the skin and adjacent mucous membranes under Dr. L. M. Ghosh at the School of Tropical Medicine, Calcutta.

The continuation of the enquiry was recommended for six months only.

(*x*) Inquiry on the problem of typing typhoid bacilli by means of Vibacteriophage and treatment of typhoid fever with type specific Vi'phage under Dr. R. G. Dhayagude and Dr. D. D. Banker at the Seth G. S. Medical College, Bombay.

This inquiry was recommended with the suggestion that Dr. Banker should confine himself to typing recently isolated strains and to the epidemiology of typhoid fever. It was suggested that work on treatment of the disease should not be pursued with a grant from the Indian Research Fund Association. Dr. K. V. Venkatraman was requested to give a short note on the lines of work on which the inquiry should be conducted.

(*xi*) Inquiry on the circulatory changes in severe infection under Dr. R. N. Chaudhuri at the School of Tropical Medicine, Calcutta.

(*xii*) Hæmatological unit under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta.

The continuation of the unit was recommended with a revised budget of Rs. 17,112/-.

(*xiii*) Hæmatological unit under Dr. J. C. Patel and Dr. Y. M. Bhende at the Seth G. S. Medical College, Bombay.

The continuation of the unit was recommended with a revised budget of Rs. 10,860/-.

(*xiv*) Polio research unit under Dr. P. V. Gharpure at the Grant Medical College, Bombay.

The Committee noted that the trained personnel of the Neuropathological unit would be transferred to this unit and recommended that Mr. C. S. Swaminathan, Chemist, should be promoted as research assistant in the scale Rs. 160-10-330.

(B) The Committee did not recommend the continuation of the following inquiries :—

(*i*) Inquiry into erythroblastosis fœtalis and the Rh factor under Lieut.-Colonel S. D. S. Greval at the School of Tropical Medicine, Calcutta.

(*ii*) Inquiry on the associated vaccine for prophylactic immunization against typhoid, paratyphoid, cholera, dysentery and tetanus by a single inoculation under Dr. J. C. Ray at the Indian Institute for Medical Research, Calcutta.

(*iii*) Clinical trials with the drug 'V-187' under Dr. N. K. Sahiar and Dr. N. M. Purandare at the K. E. M. Hospital, Bombay.

The Committee noted that clinical trials with the drug 'V-187' could not be carried out as the drug was not received from the Medical Research Liaison Committee, London. The Committee recommended that if the drug was received in the course of the year, fresh proposal should be submitted for consideration of the Committee.

(*iv*) Inquiry on the histological examination of the brain of *Microchiroptera taphozous Melanopogan* with special reference to auditory tract and medial geniculate body under Dr. (Miss) L. Chacko at the Christian Medical College, Vellore.

(C) The Committee noted that the following inquiries would terminate on the 31st March, 1950 :—

(*i*) Inquiry on the effects of vitamins B and C in diabetes under Dr. S. Banerji at the Presidency College, Calcutta.

(*ii*) Inquiry to investigate allergic diseases and significance of endogenous allergens as regards ætiology, diagnosis and treatment of allergic diseases under Dr. J. C. Ray at the Indian Institute for Medical Research, Calcutta.

(*iii*) Inquiry on the neurogenic factor in the production of traumatic shock and its treatment by the alteration of cerebro-spinal fluid electrolytes under Dr. P. K. Sen at the Seth G. S. Medical College, Bombay.

Item 2.—*To consider the report of the meeting of the Therapeutic Trials Sub-Committee held in New Delhi on the 23rd November*, 1949.

The report of the Therapeutic Trials Sub-Committee was adopted and is appended to this *report* (*Appendix I*).

Item 3.—*To consider the report of the meeting of hæmatological workers held in New Delhi on the 24th November*, 1949.

The report of the meeting of hæmatological workers was adopted and is appended to this *report* (*Appendix II*).

Item 4.—*To consider the report of the meeting of the Cirrhosis Sub-Committee held in New Delhi on the 24th November*, 1949.

The report of the Cirrhosis Sub-Committee was adopted and is appended to this *report* (*Appendix III*).

The Committee recommended that Dr. M. V. Radhakrishna Rao should be appointed as a member of the Sub-Committee.

Item 5.—*To consider the report of the meeting of the Typhus Sub-Committee held in New Delhi on the 24th November*, 1949.

The report of the Typhus Sub-Committee was adopted and is appended to this *report* (*Appendix IV*).

The Committee recommended that as there was only one inquiry on typhus, the Sub-Committee on Typhus be discontinued.

Item 6.—*To consider new proposals on clinical research.*

(A) The Committee recommended the following new inquiries :—

(*i*) Studies on the role of fungi in pulmonary diseases under Dr. P. N. Wahi at the Medical College, Agra.

The Committee recommended a sum of Rs. 3,000/- only for contingencies, purchase of *x*-ray films, etc.

(*ii*) Investigation on the cultivation of *Entamœba histolytica* in a bacteria-free medium under Dr. J. C. Ray at the Indian Institute for Medical Research, Calcutta.

The inquiry was recommended for one year only.

(*iii*) Inquiry on the relationship of pancreas to the content formation of lipocaic and ultimately the mode of its action in the control and regulation of fat metabolism under Dr. J. C. Sachdev at the Mahatma Gandhi Memorial Medical College, Indore.

The Committee recommended a sum of Rs. 400/- only for contingencies.

(*iv*) Epidemic dropsy inquiry under Dr. S. N. Sarkar at the Campbell Medical College, Calcutta.

Dr. C. G. Pandit, Secretary, I.R.F.A. informed the Committee that even though the inquiry was not included in the agenda, as it was received very late, considering the urgency of the problem and the experience of Dr. Sarkar in these lines, he considered it appropriate that the Clinical Research Advisory Committee should consider this inquiry which in ordinary course would have come before the Committee. The Committee, after considering in detail the programme, recommended the inquiry; and further suggested that the Governments of West Bengal and Bihar should be requested to contribute an amount at least equal to the grant sanctioned by the Indian Research Fund Association for this inquiry as the problem is of primary importance to the two Provinces.

(*v*) Inquiry into seminal stains under Lieut.-Colonel S. D. S. Greval at the School of Tropical Medicine, Calcutta.

The inquiry was recommended for one year only.

(B) The following new inquiries were not recommended :—

(*i*) Inquiry on the cytologic diagnosis of cancer under Dr. V. S. Mangalik at the King George's Medical College, Lucknow.

(*ii*) Inquiry on the action of antibiotics on microbial enzymes under Dr. B. N. Ghosh at the University College of Science & Technology, Calcutta.

(*iii*) Inquiry on acetylation in relation to development of tumours in rats under Dr. P. S. Sarma at the Biochemical Laboratory, University of Madras, Madras.

(*iv*) Inquiry on the specific de-allergization treatment by method of skeptophylaxis in cases of food and drug allergies under Dr. J. C. Ray at the Indian Institute for Medical Research, Calcutta.

(*v*) Determination of age in Northern Indians from a study of the ossification of the epiphyses of long bones under Dr. G. S. Sarin and Dr. N. Das at the Irwin Hospital, New Delhi.

The Committee did not recommend the inquiry in the form presented, and suggested that the sponsors should get in touch with the Secretary, I.R.F.A. for the manner in which the proposal should be submitted next year.

(*vi*) Inquiry on chemotherapy of tuberculosis under Dr. U. P. Basu at the Bengal Immunity Research Institute, Calcutta.

(C) The following inquiry was withdrawn by the sponsor :—

Studies of diabetic ketosis under Dr. S. Banerji at the Presidency College, Calcutta.

Item 7.—To consider the reports of work done submitted by the I.R.F.A. research fellows on the work done by them under the Association.

The Committee decided that members should communicate their comments on the reports submitted by the research fellows to the Secretary, I.R.F.A.

The Committee further resolved that a Sub-Committee consisting of :—

Dr. R. N. Chaudhuri (*Convener*)
Dr. R. N. Cooper
Dr. C. R. Das Gupta
Dr. P. N. Wahi
Dr. J. C. Patel

be appointed to go into the reports of the research fellows engaged in clinical research and submit its report to the Clinical Research Advisory Committee.

Item 8.— The Committee recommended that as there were no Regional Advisory Committees in the northern provinces, an adviser in each of the following places be appointed with a view to advise research workers and fellows as to the manner in which they should draw up their schemes for submission to the Indian Research Fund Association for grant-in-aid :—

1. Agra.
2. Amritsar.
3. Delhi.
4. Lucknow.
5. Patna.

Item 9.— The Committee recommended that on the termination of the inquiries, workers who ceased to be on the staff of the institution should be allowed, under the conditions normally applicable, access to their own record and data with a view to publication of the work done by them under the auspices of the Indian Research Fund Association.

Item 10.—The Committee recommended that workers should be allowed to state the choice of the journal in which they would like their work to be published in case the work was not considered suitable for publication in the *Indian Journal of Medical Research.*

APPENDIX I.

REPORT OF THE MEETING OF THE THERAPEUTIC TRIALS SUB-COMMITTEE OF THE CLINICAL RESEARCH ADVISORY COMMITTEE.

Held in Committee Room, Central Secretariat, North Block, New Delhi, at 2-30 p.m. on the 23rd November, 1949.

MEMBERS PRESENT.

Dr. M. G. Kini.	(*Chairman*).
Lieut.-Colonel Jaswant Singh.	
Dr. B. Mukerji.	
Dr. J. C. Patel.	(*Secretary*).

VISITORS.

Dr. A. C. Banerjea.
Group-Captain D. N. Chakravarti.
Dr. (Miss) S. Chaudhuri.
Brevet-Colonel R. N. Chopra.
Dr. C. R. Das Gupta.
Dr. R. N. Cooper.
Dr. J. C. Gupta.
Major S. L. Kalra.
Lieut.-Colonel D. P. Nath.
Dr. D. N. Roy.

1. *To examine further replies to the questionnaire which was issued to all hospitals in India asking for their co-operation in the conduct of clinical trials of new remedies.*

The Sub-Committee perused the replies received from various hospitals in India in regard to their co-operation in the conduct of clinical trials of new remedies under the auspices of the Indian Research Fund Association and recommended that the Secretary, I.R.F.A., be requested to prepare a list of hospitals where full facilities were available for the conduct of clinical trials of new remedies.

2. *To consider the report of clinical trials with 'Neochin' submitted by the Director, Malaria Institute of India, Delhi.*

The Sub-Committee considered the report on the clinical trials with 'Neochin' submitted by the Director, Malaria Institute of India, Delhi, and recommended that a copy of the report should be sent to the manufacturer of the drug. The Sub-Committee further recommended that the report should be published.

3. *To consider whether clinical trials of Ayurvedic and Unani medicines be undertaken by the Indian Research Fund Association.*

The Sub-Committee decided that clinical trials should be undertaken of only those Ayurvedic and Unani medicines whose composition or derivation was from a single plant or from a single mineral ingredient and that in the case of those medicines with combined substances such clinical trials will be considered on the merits of each preparation. The Sub-Committee also recommended that in view of the fact that medicines prepared under the Ayurvedic and Unani systems are supposed to be for oral treatment and not in the form of injections, any injectible preparation of these medicines should come under the control of the relevant section of the Drugs Act. The Sub-Committee recommended that this suggestion be brought to the notice of the Drugs Technical Advisory Board of the Government of India.

4. *To consider a request from Dr. Hakim Rafi-Uddin Khan of Bareilly regarding carrying out of clinical trials with 'Malaria Cure'.*

The Sub-Committee decided that Dr. Hakim Rafi-Uddin Khan should be requested to send a plant sample of the ingredient 'Jeea', viz. roots, stems, barks, leaves and flowers, etc., to the Secretary, I.R.F.A. On receipt, these should be forwarded to the Central Drugs Laboratory, Calcutta for identification and isolation of the active chemical constituents of the ingredient. It was decided to defer the question of carrying out clinical trials of the medicine till the information regarding the active chemical constituents of the plant 'Jeea' was available.

5. *To consider a request from Shree Shankar Bhai G. Patel of Padra regarding carrying out of clinical trials with 'Atomized Mica' in the treatment of tuberculosis.*

The Sub-Committee recommended that in view of the fact that the preparation 'Atomized Mica' was an injectible Ayurvedic drug, the compound composition of which was not known, clinical trials of the drug could not be carried out. Ayurvedic pharmaceuticals were not supposed to be in injectible forms.

6. *To consider a request from Dr. J. R. Goyal of Delhi regarding carrying out of clinical trials with 'Methchin' in the treatment of malaria.*

The Sub-Committee noted that trials had already been carried out on the efficacy of the drug 'Methchin' in the treatment of malaria by the Director, Malaria Institute of India, Delhi, and it was found that the drug was ineffective against malaria. In view of this, the Sub-Committee recommended that clinical trials with this drug need not be carried out.

7. *To consider a request from Messrs. Raptakos Brett & Co., Ltd., Bombay, regarding carrying out of clinical trials of their product 'Digitoxin-RB'.*

The Sub-Committee recommended that Messrs. Raptakos Brett & Company should be requested to send samples of the drug to the Secretary, I.R.F.A., who in turn should send it to the Director, Central Drugs Laboratory, Calcutta, for carrying out pharmacological tests to find out whether it is a single known substance, in which case the clinical trials are not necessary as the properties of Digitoxin are well known.

8. *To note that Mr. Abdur Rasheed Khan of Bangalore had been informed by the Secretary, I.R.F.A. that clinical trials of his remedy 'Pyorrhœa Cure Medicine' could not be undertaken as he was not prepared to disclose the composition of the remedy.*

The Sub-Committee agreed with the action taken by the Secretary, I.R.F.A., in informing Mr. Abdur Rasheed Khan of Bangalore that clinical trials of his remedy 'Pyorrhœa Cure Medicine' could not be undertaken as he was not prepared to disclose the composition of the remedy.

9. *To note that Messrs. The British Drug House (India) Ltd., Bombay, have withdrawn their request for the conduct of clinical trials with their drug 'Myanesin'.*

The Sub-Committee noted that Messrs. The British Drug House (India) Limited, Bombay, have withdrawn their request for clinical trials with their drug 'Myanesin'. Colonel S. Narain of Army Headquarters, New Delhi, placed before the Sub-Committee a note on the use of 'Myanesin' as a muscular relaxant. The Chairman, on behalf of the Sub-Committee, thanked Colonel Narain for carrying out clinical trials on the use of 'Myanesin' and for his most interesting information. It was recommended that the report should be published.

10. *To consider a request from the Indian Chemical Manufacturers' Association, Calcutta, for co-option of one of their members to the Therapeutic Trials Sub-Committee of the Indian Research Fund Association.*

The Sub-Committee, after a very careful consideration of the request made by the Chemical Manufacturers' Association, Calcutta, for representation on the Sub-Committee, felt that it was not desirable to have any representative of commercial organizations on the Therapeutic Trials Sub-Committee as a member. They, however, recommended that a representative of such commercial organization may be invited to present his case at a meeting of the Committee, whenever the question of carrying out clinical trials of the drugs submitted by that organization is considered.

11. *To consider a letter from Messrs. Raptakos, Brett & Co., Ltd., Bombay, regarding the introduction of Animal Preservation Bill in Provinces and its effect in the production of liver extracts in the country.*

The Sub-Committee were unanimously of the opinion that the manufacture of liver extracts and glandular products deserved protection and encouragement. They, however, decided that, before definite proposals are formulated on the subject, the Secretary, I.R.F.A., should be requested to obtain information on the following points :—

(*a*) Amount of liver extracts and glandular products manufactured in India.

(*b*) Amount of liver extracts and glandular products imported from abroad.

(*c*) Amount of liver and glands available to manufacturers for biological preparations from municipal slaughter-houses in the various Provinces of India.

12. *To consider the desirability of taking a deposit from firms with their request for clinical trials and to decide the amount.*

The Sub-Committee decided that a nominal fee of Rs. 25 (non-refundable) should be charged to the firms applying for clinical trials of all their product. This amount should be sent to the Secretary, I.R.F.A. along with the application for clinical trials.

13. *To note that samples of the lemon-grass oil preparations have not been received by the Indian Research Fund Association from the Indian Institute of Science, Bangalore, for clinical trials.*

Noted.

14. *Any other business.*

(*a*) The Sub-Committee considered a report submitted by Dr. J. C. Patel on the clinical trials of 'Aureomycin in the treatment of typhoid fever', which was undertaken on behalf of the Sub-Committee and resolved that the report be recorded, published and a copy should be sent to the manufacturer.

(*b*) The Sub-Committee considered the desirability of fixing a definite amount as the charge of the Indian Research Fund Association for undertaking clinical trials of liver extracts. After considerable discussion on the subject, it was decided that in order to encourage the carrying out of clinical trials of every batch of liver extract manufactured in the country by different firms, a fixed fee of Rs. 250 per trial should be charged for the conduct of clinical trials by the hæmatological units. Some members of the Sub-Committee recommended that a fee of at least Rs. 1,000 was desirable, but the Committee felt that the figure of Rs. 250 per trial would for the present appear reasonable and would encourage manufacturers in submitting liver extracts for trials. It recommended that the position should be reviewed next year in the light of the experience gained.

(*c*) The Sub-Committee then considered a letter from the Secretary, Indian Tariff Board, Bombay, to the Secretary, I.R.F.A. asking for certain information in regard to the manufacture of liver extracts in India. After discussion of the various points raised in that letter, the Sub-Committee decided that information on the following lines should be communicated to the Secretary to the Tariff Board :—

1. *Process of manufacture.*—Liver extracts are manufactured through a standard process.
2. *Specifications and quality of the indigenous products.*—Livers from slaughtered healthy animals are used in the manufacture of these extracts.
3. *Estimated annual demand in the country.*—Information regarding the amount of liver extracts imported into this country from abroad may be obtained from the Ministry of Commerce of the Government of India. The Indian Chemical Manufacturers' Association may be asked to furnish information regarding the amount of liver extracts manufactured in India. These two together will constitute the estimated demand of liver extracts in India.
4. *Actual production in India for the years* 1946, 1947, 1948 *and from January to October* 1949.—This information may be obtained from the Indian Chemical Manufacturers' Association.
5. *Handicaps, if any, encountered by the industry.*—The main hurdles in the way of development of this industry are :—

(*a*) Lack of standardization ;

(*b*) Insufficiency of the basic indigenous materials, viz. liver.

6. *Steps, if any, to be taken for the development of the industry.*—In the opinion of the Sub-Committee, the liver extract industry should be afforded all facilities and encouragement. Special consideration should be given to them when the bill banning the slaughter of animals (Animal Preservation Bill) is brought into operation by the various Provincial Governments.

7. *Your views on the protection or State assistance which may be given to the industry.*—The Sub-Committee is of the opinion that, in addition to its suggestions made in No. 6 above, the following additional steps are highly desirable :—

   (*a*) Adequate arrangements for the standardization of liver extracts manufactured in India. In this connection the Committee wishes to draw the attention of the Tariff Board to the hæmatological units set up by the Indian Research Fund Association for the purpose.

   (*b*) Adequate arrangements for the transport of healthy livers from the various municipal slaughter-houses in the country to the centres of manufacture. The local supply of these livers to the industry is far from adequate and it is, therefore, essential that hygienic arrangements should be made for the transport of livers from other parts of the country.

   (*c*) In order to give the necessary fillip to the indigenous liver extracts industry, imports from abroad of liver extracts should be restricted, as far as possible, to the minimum requirements possible.

APPENDIX II.

REPORT OF THE MEETING OF THE HÆMATOLOGICAL WORKERS.

Held in the Central Secretariat, North Block, New Delhi, at 2-30 p.m. on the 24th November, 1949.

MEMBERS PRESENT.

Dr. C. R. Das Gupta.
Dr. J. C. Patel.

VISITORS.

Dr. R. K. Anderson.
Dr. (Miss) A. Benjamin.
Dr. (Miss) S. Chaudhuri.
Dr. J. C. Gupta.
Dr. V. S. Mangalik.
Dr. B. Mukerji.
Dr. (Miss) S. Pandit.
Dr. R. Subramaniam.

The Chairman at the outset explained the genesis of the meeting of hæmatological workers and hæmatological units.

1. *To consider the progress of work done during* 1949-50 *by* :—
   - (*i*) Hæmatological unit under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta.
   - (*ii*) Hæmatological unit under Dr. J. C. Patel and Dr. Y. M. Bhende at the Seth G. S. Medical College, Bombay.

The progress of the work done at the hæmatological unit, Calcutta, was considered in detail. In the discussion it was brought out that if an orange or a lemon is taken along with iron (administered orally) better results were observed clinically than when iron is taken alone.

It was noted that the hæmatological unit at Bombay had not yet started functioning.

2. *To consider future programme of hæmatological work to be undertaken during* 1950-51.

It was decided that the above two units should be continued:

The future programme as elaborated by these two units was recommended. In the discussion it was brought out that a causation of anæmia occurring in surgical practice associated with infection should be taken up for investigation at a later stage when more funds were available.

3. *To decide the desirability of integrating research scholars, if any, who have applied to the Indian Research Fund Association on hæmatological problems in these two units.*

The hæmatological workers decided to recommend that research scholars, if any, who have applied to the Indian Research Fund Association on hæmatological problems should be attached and integrated in these two units.

4. *To consider the feasibility oj appointing a Sub-Committee to go into the question of :—*

(*a*) Manufacture and standardization of hæmatological instruments.

(*b*) Standardization of technique.

(*c*) Standardization of nomenclature.

(*a*) It was recommended that manufacture and standardization of hæmatological instruments should be taken up in India. Attention of the Indian Standards Institute, Delhi, National Physical Laboratory, Delhi, and the Glass and Ceramic Committee of the Council of Scientific & Industrial Research should be drawn to its urgent need. It was further recommended that hæmatological workers of the Indian Research Fund Association should be consulted in this matter when this question comes up for consideration. For the present it was recommended that the hæmatological unit at Calcutta should undertake standardization of hæmatological instruments.

(*b*) and (*c*) The approved technique of some of the commonly used methods, e.g. estimation of hæmoglobin, enumeration of red cells, white cells, reticulocytes and platetest, etc., will be incorporated in an appendix which will be made available at a later date.

5. *To consider the possibility of giving training in hæmatology in the hæmatological units.*

It was recommended that training be given in hæmatology in these units. Details regarding the fee, etc., will be worked out later in consultation with the Secretary of the Indian Research Fund Association.

APPENDIX III.

REPORT OF THE MEETING OF THE CIRRHOSIS SUB-COMMITTEE.

Held in Committee Room 'A', Central Secretariat, North Block, New Delhi at 10-00 a.m. on the 24th November, 1949.

Members present.

Dr. V. R. Khanolkar.
Dr. S. W. Hardikar.
Dr. P. Kutumbiah.
Dr. V. R. Naidu.
Dr. V. N. Patwardhan.
Dr. P. N. Wahi.

Visitors.

Dr. S. T. Achar.
Dr. (Miss) S. Chaudhuri.
Brevet-Colonel R. N. Chopra.
Dr. R. N. Cooper.
Dr. P. V. Gharpure.
Major S. L. Kalra.
Dr. K. V. Krishnan.
Dr. V. S. Mangalik.
Colonel S. Narain.
Dr. J. C. Patel.
Dr. (Miss) H. Patil.
Dr. C. K. Prasada Rao.
Dr. M. V. Radhakrishna Rao.
Dr. R. Sanjiva Rao.
Dr. G. L. Sharma.
Professor V. Subrahmanyan.
Dr. R. K. Vishwanathan.
Dr. K. V. Venkatraman.

Item 1.— To confirm the report of the meeting of the Cirrhosis Sub-Committee held in Bombay on the 28th March, 1949.

Certain suggestions were offered and accepted towards the formulation of the *pro forma* clinical case sheet to be used in the liver-cirrhosis investigations.

1. Particulars regarding information from the patient's relatives if the patient had suffered from conditions described in *Annexure A*. This *Annexure* will be prepared by Dr. S. T. Achar and Dr. V. N. Patwardhan.
2. If the case sheet is published by the Indian Research Fund Association common English names for Indian food items may be included in the table under dietetic investigation on page 1.

3. The opinion of a statistician before the final draft of the *pro forma* may be obtained.

Item 2.— To consider the progress of work on cirrhosis of liver in India during 1949-50, along the lines recommended by the Sub-Committee at their meeting on 28th March, 1949.

A. *Inquiry on the role of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay.*

The following comments on the inquiry were made :—

1. *Dr. Wahi.*—In his experience the response of rats to low-protein diets differs in different age groups. He has found necrosis in rats below 50 gms. (4 weeks in age), whereas adult rats developed fatty infiltration which leads to diffuse hepatic fibrosis.

2. *Dr. Patwardhan.*—(*a*) The dose of tocopherol given to rats appears to be too high since the daily needs are in the region of 1 mg. per day.

   (*b*) The work on lipoid-P is interesting and should be pursued. The conclusions reached in the present work have, however, been based on the assumption that there is a constant level of different phospho-lipoids in the lipoid-P fraction. It may be so in normals, but in disease it may be possible that this proportion changes, in which case lipoid P×25 may not give the correct figure for phospho-lipoids. Hence it is desirable to determine the quantities of individual P-lipoids if possible.

   (*c*) Recent information suggests that the source of yeast may make a difference in the production of necrosis in rats fed with yeast protein.

Recommendation.—The Sub-Committee recommends the continuation of the inquiry with a modified budget.

B. *Clinical and biochemical investigations on certain aspects of infantile hepatic cirrhosis under Professor V. Subrahmanyan and Dr. V. R. Naidu at the Central Food Technological Research Institute, Mysore, and Sri Krishnarajendra Hospital, Mysore.*

The following comments were made :—

1. Dr. S. W. Hardikar said that the inquiry was getting away from its main objective and was too diffuse in its approach and hence he suggested that it was essential that the inquiry should be confined to the objectives laid down by the Liver Cirrhosis Sub-Committee in March 1949.
2. Dr. Kutumbiah agreed with Dr. Hardikar. He said that the work originally assigned to Prof. Naidu was clinical and histo-pathological investigations in liver cirrhosis. He found that too much time and energy have been spent on aspects of the investigation which were outside the present scope of the inquiry.
3. Dr. Radhakrishna Rao suggested that we have no definite knowledge of the early lesions in infantile biliary cirrhosis and hence

the workers should concentrate on the liver biopsies in the early stages of the disease. He further suggested that if therapeutic trials are to be undertaken they should be carried out intensively with specific lipotropic substances on few cases instead of using a large number of cases.

4. Dr. Patwardhan pointed out that the biochemical work on the problem was essential, but judging from the report the method of approach appeared unsound and the evaluation of results uncritical. Under the present circumstances it would be desirable to discontinue this part of the work or to completely re-organize it.
5. Dr. Wahi found it difficult to agree with the histological findings reported by Dr. Naidu and suggested that in accordance with the recommendations of the Liver Cirrhosis Sub-Committee the sections may be sent to other workers for their opinion.

Recommendation.—The Sub-Committee recommended that Prof. Subrahmanyan and Dr. Naidu should restrict their work in the coming year to : (1) An evaluation of the histopathology of the liver by means of liver biopsies at different stages of infantile biliary cirrhosis and (2) different liver-function tests with reference to their diagnostic and prognostic value in infantile biliary cirrhosis.

The Sub-Committee recommended a modified budget and further recommended that the post of assistant research officer on the enquiry should not be continued after 1950-51.

C. *Attempts to produce liver damage in rats by dietary means by Dr. V. N. Patwardhan.*

Rats were fed with typical South Indian diets for prolonged periods. The animals were sacrificed at definite intervals and the amounts of methionine, cystine, crude fatty acids and total nitrogen in their livers were determined. Histological examination of livers was also undertaken. It was found that in these animals there was a progressive depletion of liver methionine and after a period of 21 months some rats showed evidence of commencing liver damage. Further reports about the type and extent of injury are awaited.

In toxic injury of the liver caused by CC_4 injection in rats, liver methionine was also found decreased. When dl-methionine was added as a supplement to the South Indian diet, the mortality in rats decreased and the level of liver methionine was found to be higher than in control animals.

The programme for the following year was (*a*) to study the mechanism of protective action exerted by methionine and (*b*) to continue work on poor South Indian diets and their probable role in dietetic liver injury.

Recommendation.—Dr. Patwardhan was asked to continue this work as a part of the Liver Cirrhosis Programme of investigations.

D. *Composite liver-function studies by Dr. P. N. Wahi.*

The object of this study is to correlate the clinical and liver-function studies with histological findings on liver-biopsy specimens. The following liver-function studies have been taken up :—

1. Thymol test.
2. Cephalin-cholesterol flocculation test.

3. Colloidal-gold test.
4. Total serum-bilirubin estimation.
5. Total and fractional plasma-protein estimation.
6. Prothrombin time and its response to vitamin K.
7. Urine-urobilinogen estimation.

For needle biopsy, Vim-Silverman and Iverson needles are being used.

Normal standards of these liver-function tests have been set up. A pattern chart (submitted) has been drawn and function profiles for each patient are made. It is hoped that it may be possible to set definite profiles for each disease.

Recommendation.—Dr. Wahi was asked to continue this work as a part of the liver-cirrhosis programme of investigations.

E. *Partition chromatography in liver disease by :—*

(*a*) *Dr. P. N. Wahi.*—A laboratory for study of amino-acid partition chromatography has been set up at Agra. Single dimensional technique has been perfected. Seventy normal cases have been studied and normal chromatogram made. In addition, nine cases of liver disease (cirrhosis and infective hepatitis) have also been studied. The main difficulty has been that it has been necessary to depend upon Dent's partition chart. It is essential that such chart should be prepared for each laboratory. The whole set costs about Rs. 3,000 and funds were not available for this purpose. It is necessary to obtain in the country small amounts of chemically pure amino-acids for preparation of standard chromatographic charts.

(*b*) *Dr. V. R. Khanolkar.*—During the last eight months the literature has been collected and technique for single and double dimensional partition chromatography perfected after a great effort spent in collecting reagents, etc.

Recommendation.—The Sub-Committee recommended that a sum of Rs. 3,000 be allotted for the purchase of amino-acids for the above work. The Secretary, I.R.F.A., be requested to obtain them and to supply them to the laboratories at Agra, Bombay and Coonoor, who have been assigned the work on partition chromatography.

F. *Clinical and histopathological studies in infantile hepatic cirrhosis by Dr. P. Kutumbiah.*

Liver biopsies have been regularly performed on cases of cirrhosis of liver. Biochemical tests of liver function are carried out on all these cases on admission. Then these patients are placed on a special diet (modified Patek) and choline chloride is given intravenously in suitable cases in addition to the diet. Serial biopsies have been carried out at monthly intervals and biochemical tests repeated. So far the work done is very promising. Specimens have been obtained and fatty accumulation and fibrosis of various magnitude and some idea of the evolution of disease is obtained. Two cases of infantile hepatic cirrhosis have come under observation and liver biopsies obtained. The work is being continued.

Recommendation.—Dr. Kutumbiah was asked to continue this work as a part of the liver cirrhosis programme of investigations.

The Sub-Committee would like to record their appreciation of the work referred to above carried out at Agra, Bombay, Coonoor and Vellore, without any financial aid from the Indian Research Fund Association, and which has been integrated in a country-wide investigation on liver cirrhosis.

Item 3.— To consider the programme of work to be undertaken during 1950-51.

The Sub-Committee's recommendations on work to be carried out in 1950-51 have been given under each inquiry in item 2.

Item 4.— To consider a request from Dr. J. C. Sachdev, Professor of Physiology, Mahatma Gandhi Memorial Medical College, Indore, for a grant from the Indian Research Fund Association to study the relationship of pancreas to the formation of lipocaic and ultimately the mode of its action in the control and regulation of fat metabolism.

The Sub-Committee did not recommend the inquiry *in toto.* It, however, recommended that a sum of Rs. 400 be granted to Dr. Sachdev for use as contingencies, with the suggestion that he might continue the work which he has started and submit next year the progress of the work with a fresh proposal for consideration. The Sub-Committee will give it a high priority provided the proposal is found acceptable.

Item 5.— Any other business.

The Sub-Committee recommended that the Secretary, I.R.F.A. be requested to help in procurement of certain reagents and appliances required by research workers on the problems. The workers concerned were requested to send their requirements to Dr. V. R. Khanolkar at an early date.

APPENDIX IV.

REPORT OF THE MEETING OF THE TYPHUS SUB-COMMITTEE OF THE CLINICAL RESEARCH ADVISORY COMMITTEE.

Held in Committee Room 'B', Central Secretariat, North Block, New Delhi at 2-30 p.m. on the 24th November, 1949.

Members present.

Dr. K. V. Krishnan. (*Convener*).
Major S. L. Kalra, I.A.M.C.
Dr. V. R. Naidu.
Dr. D. W. Soman.

Visitors.

Brevet-Colonel R. N. Chopra.
Lieut.-Colonel A. N. Duggal.
Lieut.-Colonel S. D. S. Greval.
Dr. M. O. T. Iyengar.
Lieut.-Colonel Kapila.
Colonel S. Narain.

The work of the Bengal typhus enquiry was discussed. Major Kalra observed that the team deserved to be congratulated on completing the problem of transmission of scrub typhus which had baffled both American and British workers previously. As regards the future plan of work of this inquiry it was emphasized that special attention should be paid to the work on mites and preparation of rickettsial antigens.

The Sub-Committee next went into the question of the future programme of research work on typhus. As a result of the discussions among others, two recommendations were made which, in the opinion of the Committee, were of highest importance and urgency. One was that intensive efforts should be made for manufacturing the various rickettsial antigens for the performance of complement-fixation tests without which it was felt correct diagnosis could not be made and also surveys for determining the extent of prevalence of typhus fevers could not be undertaken. The obtaining of antigen suspensions from U.S.A., it was felt, would be costly, time-consuming and might not be as satisfactory in all cases as antigens prepared from local strains. The second recommendation was that the study of the bionomics and life-cycle of all species of mites and ticks common in India associated with typhus should be intensified and the new species found, studied systematically so that ultimately typhus workers in other parts of India may be furnished with data helpful in the identification of species. The typhus workers in the Army are at present sending some of their specimens outside India for identification and it would be a great help if a centre is established where the mites and ticks of India could be identified and where specimens from all over India could be maintained.

The Committee placed on record their appreciation of Dr. R. A. O. Smith's gesture for a voluntary cut in his pay from Rs. 500 to Rs. 250 to enable a junior worker in entomology to be appointed to take his place after training in his special work on mites under him.

MATERNITY AND CHILD WELFARE.

1. Inquiry into the morbidity of children between the ages of 1 and 5 years (pre-school) in the Singur Health Centre area under Dr. Muktha Sen at the All-India Institute of Hygiene & Public Health, Calcutta.

The object of the inquiry was to elicit information with regard to the sickness rate and the causes of illness in this age group of children. This is expected to help in the future planning of medical care for a rural community.

A preliminary survey as to the health status of each child of this age group living in the area was done by visiting every child in the house. This gave an opportunity to study the environmental conditions of the children and also to explain to the people the object and the programme of work. The ill children were advised to come to the clinic which was held twice a week in the health centre. Since the 15th September, 1949 eight clinics have been started in the different villages and visits are made every week. Homes are also visited to examine the ill children, when necessary. The speed of work has been hampered to a certain extent due to monsoon conditions in this rural area.

The stool and blood of the ill children were examined whenever it was found necessary and possible. Thirty-six bloods and 102 stools have been examined till now.

A short note on the findings of medical examinations done of 547 children in their homes is given below :—

Distribution of children according to the three different age groups and their health status.

Age, years.	Number of children examined.	Well.	Ill.
1–2	197	66	131
2–3	187	83	104
3–4	163	74	89

Age 1 to 2 *years :* Of the total number of 197 children only 66 were found to be without complaints at the time of visit. The most important cause of illness among the remaining 131 children was cold which was seen 66 times. The next one was diarrhœa accounting for 38 visits, the third one was boils, which was found 31 times and the fourth in the order of importance was fever which was observed on 21 occasions. There was only one case of infectious disease seen, i.e. mumps.

Age 2 to 3 *years :* This group of children enjoyed better health than their younger brothers and sisters of 1 to 2 years, 83 of the 187 children were well and only 104 were ill. The diseases according to the order of importance were cold, diarrhœa, fever and boils. Only fever and boils have interchanged places. Other diseases take the same place as the previous group. There was no case of infectious disease in this group.

Age 3 to 4 *years:* These children did not differ very much from the previous group with regard to their state of health. The important diseases were the same as above. There was one case that had been diagnosed as typhoid.

Development : Interest was taken with regard to dentition, specially with the age group 1 to 2 years. Two children had no teeth, showing that they cut no tooth during the whole infancy. Four had only two teeth and 16 were as precarious as to cut 20 teeth within the age of two years. Among the 2 to 3 years group one had only eight teeth, nine had 10 teeth and only 112 had the full complement of 20 teeth.

As to the motor control of the age group 1 to 2 years, two could only sit and four could only crawl.

2. Inquiry into the vital losses of pregnancy and infancy in a rural population under Dr. Muktha Sen and Mr. K. K. Mathen at the All-India Institute of Hygiene & Public Health, Calcutta.

The object of the inquiry was to obtain reliable information with regard to the vital losses of pregnancy and infancy in the rural areas of Bengal.

The material used for the inquiry are the records kept by personnel of the Maternity and Child Welfare Section of the Singur Health Centre from 1944 to 1948. It covers the pregnancy in the three Union Boards of Singur, Borah and Begampur situated in the Hooghly district. The expectant mothers were contacted by the staff both in the homes and in the clinics and detailed records were maintained by the follow up.

The inquiry is in progress. A short note on the analysis done of the 1,592 pregnancy records is given below :—

Result of pregnancy.—Of the 1,592 pregnancies 1,483 resulted in live births. There were 174 still-births and 26 abortions. This gives a still-birth rate of roughly five per 100 live births and an abortion rate of two per 100 live births. The still-birth rate seems to be a fairly correct one, while that of abortion is rather an underestimate. The number of pregnant women contacted before three months of gestation are generally very few and most of the abortions take place within this period and hence most probably many are missed.

Place of delivery.—The health centre has a Maternity Home with four beds for normal delivery cases and there are four maternity beds in the General Hospital for abnormal delivery cases. In spite of these facilities most of the deliveries are conducted in the homes. Of the total number of deliveries 1,427 were in the homes, 81 in the Maternity Home and six in the Hospital.

Nature of delivery.—The percentage of abnormals are not very high. There were only five cases that needed forceps delivery and two others needed some other interference. The rest were all normal.

Maturity.—The live births were divided into full-term and premature. Diagnosis of prematurity was done either by noting the height of the uterus or by the clinical signs seen in the child. More reliable standards like birth-weight and the last date menses could not be used as it is not possible to elicit this information from the rural women. Judging from the above standard 1,309 were classed as full-terms and 174 as prematures of the total 1,483 live births.

Infants' longevity.—Of the total live births there were 100 infant deaths. Of these 85 died within one month of life or in the neo-natal period. The rest 103 died between the ages of 1 and 12 months. The most important cause of death was prematurity which accounted for 44 deaths. The next cause in the order of importance was diarrhœa which was responsible for 20 deaths. The third one was fever which had 20 deaths to its credit.

Taking the 174 premature live births, 85 were alive at the end of one year, 56 died and 28 left the area. This gives a very high infant-mortality rate of 321 per 1,000 premature births, even if all the infants that were lost contact are considered as alive up to one year. Of the 56 deaths 42 died before one month and 15 during the rest of the 11 months.

Taking the 1,309 full-term live births, 928 became toddlers and 120 died. The rest were lost contact at different ages. The mortality rate calculated as given above was 91 per 1,000 full-term live births. The number of deaths during the different age periods of infancy was more evenly distributed here than in the case of prematures. There were 33 deaths before one month, 30 from one to three months, 30 from three to six months and 27 from six to 12 months.

Mother's longevity.—Of the 1,592 women, 10 women died before the child was one year. Of these, six died either during pregnancy or before the child was one month old and so are classed as maternal death and four between one and 12 months after delivery.

Parity.—Regarding the parity of these 1,592 expectant women 278 were expecting for the first time. This is about 18 per cent of the total. Of these, 47 were below the age of 15 years, 204 between 18 and 20 years, 15 between 21 and 25 years and four between 26 and 30 years.

Spacing.—The interval between the previous birth and the present was less than one year in the case of 23 women, one year to two years in 249 cases and two years to 2½ years in 383 cases.

The detailed analysis of these records and other data is being continued.

REPORT OF THE MATERNITY AND CHILD WELFARE ADVISORY COMMITTEE.

Held in the Central Secretariat, North Block, New Delhi, at 10-00 a.m. on the 22nd November, 1949.

Members present.

Dr. J. Jhirad.	(*Chairman*).
Dr. S. T. Achar.	
Dr. A. Benjamin.	
Dr. C. R. Das Gupta.	
Dr. D. M. Satur.	
Dr. Muktha Sen.	
Dr. Satya Swaroop.	
Dr. S. Pandit.	(*Secretary*).

Dr. B. C. Das Gupta was unable to be present.

Visitors.

Miss T. K. Adranvala.
Dr. N. T. Advani.
Dr. R. K. Anderson.
Dr. D. P. Bali.
Dr. A. C. Banerjea.
Lieut.-Colonel Barkat Narain.
Miss Brown (W.H.O.).
Group-Captain D. N. Chakravarti.
Dr. T. Chandrasekharaiya.
Miss M. Craig.
Lieut.-Colonel S. D. S. Greval.
Lieut.-Colonel C. K. Lakshmanan.
Colonel D. P. Nath.
Dr. Cecily D. Williams (W.H.O.).

The Chairman in her preliminary remarks expressed her regret that no proposals for new inquiries had been received. She hoped that, in view of the national importance of the subject of maternity and child welfare, efforts would be made by the members in their respective Provinces for the intensification of research in the subject.

I. Consideration of the progress of work done on inquiries sanctioned during the year 1949-50.

(*i*) *Inquiry into the morbidity of children between the ages of* 1 *to* 5 *years* (*pre-school*) *in the Singur Health Centre area under Dr. Muktha Sen at the All-India Institute of Hygiene & Public Health, Calcutta.*

The Committee reviewed the report on the work done so far and noted that during the months July to September, 523 children were examined. Dr. Muktha Sen proposed to follow up hundred children of each age group to determine the morbidity rate.

During the course of discussion it was pointed out that the information as presented in the report appeared to be rather superficial and the Committee suggested that fuller investigation including mental and physical growth of the child along with more detailed pathological examination be carried out. In view of the prevalent malnutrition amongst children of these age groups, the Committee further suggested that the standard of nutrition of each child should be carefully determined and that the classification of morbid conditions according to the international list be adopted.

The Committee recommended continuance of the inquiry and in view of the nature of work involved, agreed to the replacement of the health visitor by a doctor and the addition of a peon, as suggested by Dr. Muktha Sen. The Committee recommended that this change be implemented during 1949-50, the additional expenditure involved being met out of the anticipated savings in the current year's budget.

(*ii*) *Inquiry into the vital losses of pregnancy and infancy in a rural population under Dr. Muktha Sen and Mr. K. K. Mathen at the All-India Institute of Hygiene & Public Health, Calcutta.*

The Committee noted that the inquiry was to be completed by March 1950. It was suggested that the past obstetric history should be taken into consideration when analysing the records.

At this stage Colonel Greval, with the permission of the Chairman, again reminded the members to help in the collection of sera from mothers of erythroblastotic babies.

II. (*i*) *To consider the final reports of the inquiry on the bearing of premature and immature births on infant mortality in the City of Bombay under Dr. B. C. Das Gupta, Executive Health Officer, Bombay Municipality, Bombay.*

The Committee received the final report of the inquiry and expressed their appreciation of the work carried out.

The Committee suggested that, as some of the conclusions drawn could not be reconciled with the tabulated statements, the report should be gone into carefully by Dr. Das Gupta, Mr. Swaroop and Dr. (Miss) Pandit before publication.

(*ii*) *Inquiry on anœmia in pregnancy under Dr. R. G. Dhayagude at the Seth G. S. Medical College, Bombay.*

The Committee noted that 120 cases of anæmia in pregnancy were fully investigated ; 101 of these being classified as nutritional megaloblastic anæmia, 19 as microcytic hypochromic anæmia. The members expressed their appreciation at the thorough investigation undertaken correlating the Hæmatological and Biochemical findings. The conclusion that the presence of the megaloblast either in the marrow or peripheral circulation being essential to the diagnosis of this type of anæmia was not accepted by some of the members, and, as Dr. Dhayagude was not present, it was decided to refer their opinions to him for clarification.

III. To receive reports on the action taken with regard to items III and VI of the minutes of the meeting of the Maternity and Child Welfare

Advisory Committee held on the 29th November, 1948, viz. definition and causes of maternal deaths ; and formation of Advisory Boards in large municipalities.

(*i*) *Definition and causes of maternal deaths.*

Dr. Pandit reported that the definition adopted at the last meeting had been circulated but with a view to effect a better classification of the causes of maternal deaths, she had drawn up *pro formas* for classifying maternal deaths : (*a*) those certified or attended by doctors and (*b*) others. The forms were adopted and recommended for circulation to the Provinces.

(*ii*) *Formation of Advisory Boards for the investigation of maternal deaths.*

The Secretary reported that out of 111 municipalities who were addressed in the matter, 31 had formed Advisory Boards, but in the absence of a proper directive they were not functioning effectively. The Committee authorized the Chairman and Secretary to draw up a suitable note for their guidance.

IV. Dr. Cecily Williams, Consultant, World Health Organization, with the permission of the Chairman, informed the members that a Maternity and Child Welfare Team of the WHO was already in India and that, at the suggestion of Dr. Pandit, the WHO was considering the feasibility of convening a maternity and child welfare symposium to discuss the various problems in this field and to guide the team in its work. She hoped that the Provinces and Municipalities would depute workers to attend this conference.

V. Dr. S. T. Achar addressed the members and spoke of the high incidence of nutritional dystrophies amongst the children of Madras. He said that the children responded to intensive protein therapy but relapses were fairly common. He suggested that the Committee should recommend for a special allotment of milk and milk products for children which was agreed to.

VI. At the suggestion of Dr. C. R. Das Gupta the Committee decided that a list of workers and institutions where further research in maternity and child welfare could be undertaken should be prepared and that suitable subjects for research be recommended.

He next moved the following resolution which was seconded by Dr. Satya Swaroop :—

> ‘ This Committee recommends that the Chairmen of Advisory Committees, who were not members of the Scientific Advisory Board, be co-opted for the specific purpose of explaining their Committees’ recommendations’.

The resolution was unanimously adopted for recommendation.

VII. Group-Captain Chakravarti (Air Force) requested the Committee to consider the question of undertaking work on family planning. The Committee, however, felt that they could only consider proposals for research on the suitability of various appliances devised for such purpose.

VIII. The Chairman suggested that proposals for inquiries into infant mortality on the lines detailed in the ‘ Memorandum on the conduct of an inquiry into infant mortality ’ be invited.

FILARIASIS.

1. Filariasis inquiry under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta.

Screening tests.—This work was started last year. During the present year, eighteen compounds were tested against *microfilariæ in vitro*, fifteen of which were received from Dr. K. Venkatraman, Director of the Department of Chemical Technology, University of Bombay and three compounds from other sources.

Venous blood of patients and out-patients with filarial infection was drawn at 10 p.m. and mixed with 0·3 per cent solution of sodium citrate in normal saline. A little heparin was added and then the blood was hæmolysed with saponin solution and the *microfilariæ* were concentrated by repeated centrifugalization. They were finally suspended in a mixture of three parts of hydrocele fluid and one part of Ringer-Locke solution (containing 0·4 per cent glucose) and the suspension was so made that each drop contained at least fifty *microfilariæ*.

The compounds were dissolved in Ringer-Locke solution and different dilutions were made. In easily soluble compounds dilutions were started from 1 in 4,000. In sparingly soluble compounds the initial dilution made was 1 in 40,000. Addition of extra alkali to help solution was avoided as strong alkaline solutions were found to be lethal to *microfilariæ* in the controls. The pH of the Ringer-Locke solution used was adjusted to 7·4 in all cases. The *microfilariæ* in the controls were all suspended in three parts of hydrocele fluid and one part of Ringer-Locke solution.

The experiments were put up by midnight. During the night readings were taken every two hours and in the following day and night once every three to four hours until all the *microfilariæ* in the experimental tubes as well as in the controls were dead as judged by cessation of movement.

'Lethal concentration' of the drugs was determined when most of the *microfilariæ* were inactive in the solution whereas all the *microfilariæ* were active in the controls. The time during which this happened, correlated with the dilution of the drug, and seemed to give a true picture of the activity of the drug in inhibiting the movement of the *microfilariæ*.

The tests with many of the drugs were repeated. The results are shown in the Table :—

TABLE.

Serial number.	Drugs.	Lethal concentration.	Time in hours.
1.	4-amino-salicylic acid	1-4,000	16
2.	Di-sodium salt of 3 : 3^1-di-hydroxy-azobenzene-4 : 4^1-carboxylic acid	1-8,000	16
3.	Astraphloxine FF	1-64,000	17
4.	Salt of astraphloxine FF and potassium-guaiacol sulphonate	1-128,000	14
5.	Salt of astraphloxine FF and sodium lauryl-p-toluidide-2-sulphonate	1-256,000	14
6.	Sulphanilamide salt with naphthalene-sulphonic acid	1-128,000	22
7.	Sulphanilamide salt with phenol-p-sulphonic acid	1-128,000	22
8.	Propyl ester of p-carboxy-stibonic acid	1-128,000	15
9.	p-acetamide-stibonic acid	1-640,000	12
10.	p-laurylamide-stibonic acid	1-80,000	13
11.	p-phenetidine-antimonyl tartarate	1-40,000	21
12.	Citrinin	1-40,000	15
13.	p-carboxy-stibonic acid	1-64,000	16
14.	Soluble antimonial	1-16,000	16
15.	4-laurylamid-2-methoxy-stibonic acid	1-160,000	14
16.	Paludrine acetate	1-64,000	13
17.	M. & B. 1364	1-256,000	19
18.	M. & B. 736	1-128,000	18

Intradermal tests.—No work on intradermal tests could be done as the antigen for the work could not be obtained from the King Institute, Guindy, Madras.

2. Inquiry to test the usefulness of intradermal and other immunological tests in the diagnosis of early filariasis under the Director, King Institute, Guindy, Madras.

A distilled-water extract of adult *Conispiculum guindiensis* was used in the tests during the period under report.

The method of preparation was the same as that described in last year's report except that distilled water was used for the extraction of the dried residue in place of normal saline.

The nitrogen value of this extract was 0·29 mg. nitrogen per c.c. as again 0·46 mg. per c.c. with the saline extract.

The tests were carried out in the Saidapet Government Dispensary, Madras from among 511 cases grouped, as follows on the basis of a careful appraisal of the history, physical examination and examination of the blood for *microfilariæ* :—

	Number of cases.
Clinical filariasis	335
Probably filarial	42
Control cases	134
Total	511

The presenting manifestations of the disease were :—

	Number.	Per cent.
Œdema of lower extremity (one or both legs)	207	55
Œdema of upper extremity (one or both hands)	37	10
Œdema of foot and hand or feet and hands	10	2
Lymphangitis or lymphadenitis or both with or without pyrexia	57	15
Epididymo-orchitis	47	13
Urticaria	4	1
Aching sensation in extremities	9	2·5
Lymph-scrotum	2	0·5
Mastitis	4	1

The tests were done by intradermal injection of 0·01 c.c. of the antigen on the volar aspect of the forearm at two or three spots separated from each other by a distance of 5 cm. A wheal resulted reaching its maximum in 15 minutes.

The mean dimension of the wheal in millimetres is given in Table I :—

TABLE I.

Type of case.	Number tested.	1 : 100,000	1 : 10,000	1 : 1,000
Clinically filarial	149	4	8·3	14
	20	3	3	3
Controls	19	2·4	2·7	3
	10	6	11	16
Probably filarial	4	5	9	14

It was observed, more especially in a large proportion of clinically filarial and probably filarial cases, that there was a considerable increase in the size of the wheal with the lower dilutions of the antigen. It would appear that the relative increase in the size of the wheal on the application of a lower dilution of the antigen is more significant than the actual size. The cases in each clinical grouping have, therefore, been subdivided according to whether they show an increased size of the wheal with the lower dilution of the antigen or not.

To study if filtration of the distilled-water extract through a candle has any effect on the dermal response, a group of cases was tested with filtered and unfiltered extracts. The results showed that there is no significant difference in the action of the two extracts.

TABLE II.

Extract.	N_2 value mg. of N_2 per c.c.	Clinically filarial (19 cases):			Control cases (8 cases):		
		1:100,000	1:10,000	1:1,000	1:100,000	1:10,000	1:1,000
Unfiltered extract.	0·29	5	8	12	1	2	4
Filtered extract.	0·26	4	8	11	1	2	4

The serum of infected *Calotes versicolor* was used as an antigen and compared with the serum of uninfected *Calotes*.

The blood was collected with sterile capillary pipettes from the chambers of the heart of the *Calotes* and the serum diluted to 1 : 100 with normal saline, filtered through Seitz, tested for sterility, and if sterile, phenolized to 0·3 per cent. It was stored in the cold in ampoules of 1 c.c. The mean dimension of wheals in millimetres obtained with the use of these antigens in 159 cases is given in Table III :—

TABLE III.

	Number tested.	Uninfected *Calotes* serum.		Infected *Calotes* serum.	
		1:100,000	1:10,000	1:100,000	1,10,000
Clinically filarial	135	2	2	2·5	3
Control cases ..	19	2	2	2	2
Probably filarial	5	3	3	2	2·5

It could be seen that there is no significant reaction.

Precipitin tests.—Three guinea-pigs were immunized by nine intraperitoneal injections of 1 c.c. of *Conispiculum guindiensis* extract (saline extract 1 : 100) in a period of five weeks.

The sera of these failed to show any flocculation or precipitation with the antigen (undiluted serum and serial dilutions of serum 1 : 15, 1 : 30, 1 : 60, 1 : 120, 1 : 240, 1 : 480 being used) at varying intervals during the course of immunization and also after the completion of the course.

A series of 13 compounds prepared by Professor K. Venkatraman in the inquiry on ‘ Changing the properties of drugs by wetting agents ’ in the Department of Chemical Technology, University of Bombay were tested for any therapeutic action they may have in *Conispiculum guindiensis* infection of *Calotes versicolor*.

The drugs were administered in varying doses, daily for a period of seven days. Frequent observations were made on the number of *microfilariæ* present in 3 c.mm. of blood during treatment and at the end of the course.

In the case of some of these compounds the dosages administered were toxic and a certain number of animals died during treatment. The surviving animals were sacrificed and the condition of the adult worms noted. In the case of animals which died during treatment these observations were not reliable as the worms also appear to perish within four to six hours after the death of the *Calotes*.

It was noted that none of the 13 compounds tested had any effect on the number of *microfilariæ* or on adult worms.

TABLE IV.

Results showing the toxic and tolerated doses of the compounds.

Compound number.	Tolerated dose.	Toxic dose.
1	10 mg.	
2	10 mg.	
3	5 mg./kg.	10 mg./kg.
4	2-5 mg./kg.	
5	2 mg./kg.	40 mg./kg.
6	0·25 mg.	0·5 mg.
*7	0·3 c.c.	
8 and 9	0·5 mg.	1 mg.
10, 11, 12 and 13	0·5 mg.	1 mg.

Compound number.	Name.
1	Para-amino-salicylic acid.
2	Sodium salt of 3 : 3 di-hydroxy-azobenzene–4 : 4^{1}–di-carboxylic acid.
3	1 : 5 di- (N. methyl-B-di-methyl)-indoxyl △penta-diene iodide.
4	Salt of astrophloxine FF and potassium-guiacol sulphonate.
5	Salt of astrophloxine FF and sodium-lauryl-p-toluidide-2-sulphonate.
6	C≠·863: 1-ethyl-3^{1}: 6^{1}-di-methyl 2^{1}-phenyl-4^{1}-pyramide 2-cyanine chloride.
7	Salt from C≠863 and aerosol OT.
8	O-methoxy-p-acet-amido-stibonic acid.
9	O-methoxy-p-benzamido-stibonic acid.
10	Water-soluble antimonial.
11	Methylene blue and Garden olivia.
12	Methylene blue and B.D.T.
13	p. rosaniline and Nekal Bx. salt.

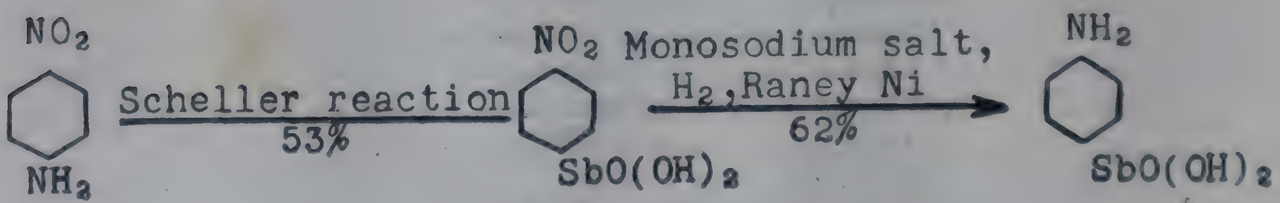
*Compound 7 was admininistered orally.

3. Inquiry on changing the physical properties of drugs by wetting agents under Dr. B. B. Dikshit at the B. J. Medical College, Poona and Dr. K. Venkatraman of the Technology Department of the University of Bombay.

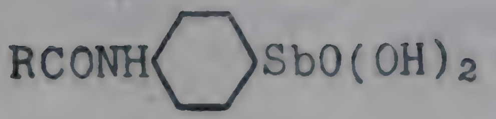
The chemical part of the inquiry, which has involved extensive exploratory work, is now making progress on the lines indicated in earlier reports. The present report outlines the preparation of thirty new compounds. Antimonials being the drugs so far commonly employed in the treatment of filariasis, two pentavalent and trivalent antimony compounds possessing marked antifilarial action have been modified in their chemical constitution by the introduction of long alkyl chains which lead to surface-activity. Thus, homologues of stibacetin and p-phenetidine-antimonyl tartarate have been synthesized. Further, nuclear substituted derivatives of stibanilic acid, which may also be regarded as derivatives of an antimony analogue of p-amino-salicylic acid, have been prepared, and it is interesting to note that one of these compounds, 4-dodecanoyl-amido-2-methoxy-phenyl-stibonic acid (IV, $R=C_{11}H_{23}$) has been found to be twice as active as 4-dodecanoyl-amido-phenyl-stibonic acid (II, $R=C_{11}H_{23}$) as judged from their microfilarial count. In view of the topical interest in 4-amino-salicylic acid (PAS) in the chemotherapy of tuberculosis, and from the more general point of view of surface-activity and chemotherapeutic action, the preparation and examination of a series of derivatives of PAS have been included in the programme of work of this inquiry. A paper describing the synthesis of PAS by a convenient procedure and a few of its derivatives has appeared in the *Proceedings of the Indian Academy of Sciences* (**29**, p. 196, 1949). Further derivatives of PAS have since been prepared.

Reports on the anti-filarial activity of the compounds now described, as well as certain compounds referred to in earlier reports, are being made separately by Dr. A. K. Mukerji, to whom our thanks are due. Grateful acknowledgment is also made to Dr. K. V. Venkatraman who has tested some of these compounds in lizard filariasis.

The parent compound of most of the pentavalent antimonials, which have found use in filariasis, is stibanilic acid. Old and new routes for the synthesis of the acid were, therefore, examined, but the recent method of Doak and Steinman (*Jour. Amer. Chem. Soc.*, p. 1987, 1946) could not be improved upon. N-substituted derivatives of stibanilic acid can then be prepared without isolating this acid from solution, after removal of the catalyst by filtration. Employing the method,

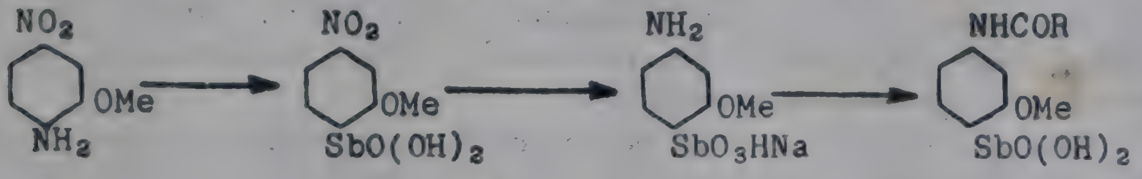


(I)



(II)

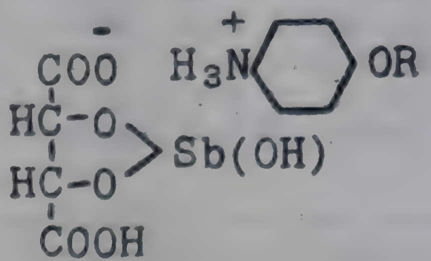
the solution obtained on reduction of (I) was shaken with dodecanoyl chloride for thirty minutes, keeping the solution just alkaline ; acidification gave p-dodecanoyl-amido-phenyl-stibonic acid (II, R=$C_{11}H_{23}$) (found : Sb, 33·2 $C_{18}H_{30}O_4NSb$ requires Sb, 27·3 per cent). N-substituted derivatives of an antimony analogue of PAS were synthetized by a similar procedure. The solution obtained on reduction of 4-nitro-2-methoxy-stibonic acid (III) (found : N, 4·1 ; Sb, 37·6. $C_7H_8O_6NSb$ requires N, 4·2 ; Sb, 37·6 per cent) was shaken with acetic anhydride and worked



(III) (IV)

up further in the usual manner to obtain 4-acetamido-2-methoxy-phenyl-stibonic acid (IV, R=Me). 4-benzamide-2-methoxy-phenyl-stibonic acid (IV, R=Ph) and 4-dodecanoylamido-2-methoxy-phenyl-stibonic acid (IV, R=$C_{11}H_{23}$) (found : Sb, 30·5. $C_{19}H_{32}O_5NSb$ requires Sb, 25·6 per cent) were similarly prepared.

Trivalent antimonials, such as fuadin and anthiomaline, are used in the treatment of filariasis, and Newton *et al.* (*Amer. Jour Trop. Med.*, **25**, p. 263, 1945) have found that p-phenetidine-antimonyl tartarate is one of the most active compounds in *Dirofilaria immitis* and *Litomosoides carinii.* They consider that it is worthy of extended experimental and clinical trial in human filariid infections. Their work also indicates that p-phenetidine-antimonyl tartarate is better than the corresponding o- and m-isomers and superior to the p-anisidine derivative in its anti-filarial activity. The synthesis of a wider range of p-alkoxy-aniline-antimonyl tartarates has now been undertaken. The general method of preparation is as follows : p-anisidine (6·15 g., 0·05 mole), tartaric acid (7·5 g., 0·05 mole) and an excess of antimony trioxide (10·2 g., 0·035 mole) in water (80 c.c.) was refluxed for three hours. While hot, the reaction mixture was filtered to remove the unreacted antimony oxide, and the filtrate cooled to obtain the crystalline salt (V, R=Me). Following the procedure



(V)

p-phenetidine antimonyl tartarate (V, R=Et) and p-N-hexyloxyaniline antimonyl tartarate (V, R=C_6H_{13}) have so far been prepared. These salts are colourless crystalline compounds soluble in water, the solubility in water decreases with increase in length of the O-alkyl group.

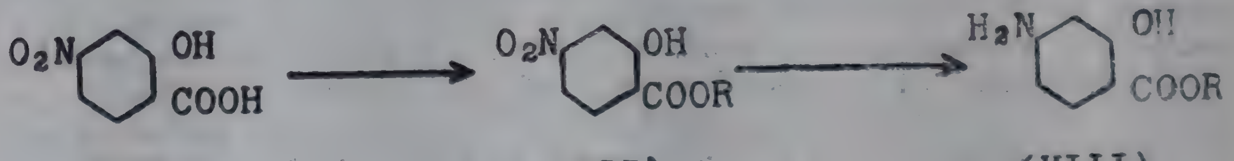
Two water-soluble complexes of antimony and catechin, differing in antimony content (one containing Sb, 29·7 and another 14·1 per cent) have been synthesized by two different methods, and work on their constitution is in progress.

The estimation of antimony in the compounds described has been carried out by Norton and Kochs' method (*Jour. Amer. Soc.*, **27**, p. 1247, 1905) as modified by Ghosh (*Ind. Jour. Med. Res.*, **16**, p. 457, 1928); organic matter is destroyed by concentrated sulphuric acid and the residual antimony is determined volumetrically by iodine. It will be observed that the antimony content of the compounds of the pentavalent series is higher than the theoretical values in common with the experience of other workers.

4-amino-4^1-lauryl-amido-di-phenyl-sulphone, m.p. 153°C. to 154°C. (found : N, 7·0. $C_{24}H_{34}O_3N_2S$ requires N, 6·5 per cent), has been prepared from the corresponding nitro compound, m.p. 166°C. (found : N, 6·2. $C_{24}H_{32}O_5N_2S$ requires N, 6·1 per cent). The corresponding stibonic acid has not yet been prepared.

The preparation of PAS is attracting wide attention, and several papers on the subject have appeared after the publication of Bhate, Panse and one of us, whose procedure via 4-nitro-salicylic acid has advantages, especially for the synthesis of derivatives of PAS. Pilot plant experiments on this process for the manufacture of PAS have been undertaken by the Department of Chemical Technology, University of Bombay, on behalf of a local firm.

The synthesis of derivatives directly from PAS is difficult because of the facile de-carboxylation of the acid. A series of esters of PAS has now been prepared from the corresponding esters of 4-nitro-salicylic acid (VI). Thus, from the methyl ester (VII, R=Me), m.p. 97°C. (found : N, 7·4. $C_8H_7O_3N$ requires N, 7·2 per cent), ethyl ester (VII, R=Et), m.p. 87°C. (Borsche, *Ann.*, **390**, p. 18, 1922, records the same m.p.) ; and n-propyl ester (VII, R=C_3H_7), a viscous oil, the following esters * of PAS were prepared by



(VI) (VII) (VIII)

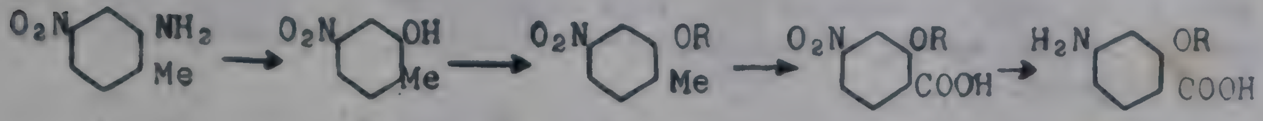
reduction in benzene solution in presence of Raney nickel with hydrogen at 40-lb. pressure : methyl ester (VIII, R=Me), m.p. 120°C. to 121 C. (Rosdahl, *Svensk. Kem. Tid.*, **60**, p. 12, 1948, records m.p. 108°C.) (found : N, 8·3. $C_8H_9O_3N$ requires N, 8·3 per cent) ; ethyl ester (VIII, R=Et), m.p. 115°C. (found : N, 7·6. $C_9H_{11}O_3N$ requires N, 7·7 per cent) ; and n-propyl ester (VIII, R=C_3H_7), m.p. 103°C. (found : N, 7·0. $C_{10}H_{13}O_3N$ requires N, 7·2 per cent).

While N-substituted derivatives and esters of PAS have been reported by various workers, little attention seems to have been paid to the preparation of ethers and O-acyl derivatives, notwithstanding the observation of

---

*After the completion of this work, these esters have also been described by Drain *et al.* (*J.C.S.*, p. 1498, 1949).

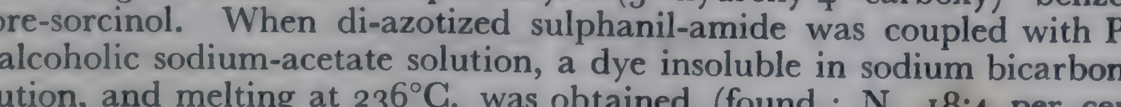
Lehmann (*Lancet*, **250**, p. 15, 1946) that PAS and its methyl ester are equally active. The synthesis of other ethers of PAS were, therefore, considered to be of interest. 5-nitro-O-toluidine prepared by hydrolysis of the di-azonium salt of 4-nitro-O-toluidine, was alkylated to



(IX) (X) (XI) (XII)

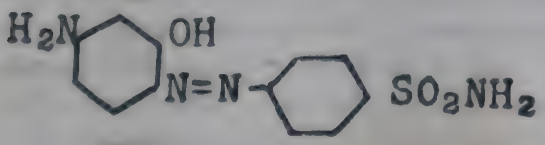
from the O-methyl ether (X, R=Me), m.p. 71°C. (Ullmann and Fitzenkam, *Ber.*, **38**, p. 3790, 1905, record the same m.p.) ; O-ethyl ether (X, R=Et), m.p. 55°C. (Spiegel *et al.*, *Ber.*, **39**, p. 3248, 1906, record m.p. 61°C.) (found : N, 7·8. $C_9H_{11}O_3N$ requires N, 7·7 per cent) ; and O-n-butyl ether (X, R=C_4H_9), m.p. 50°C. The nitro ethers were then oxidized with potassium permanganate in neutral solution to the corresponding acids : (XI, R=Me), m.p. 147°C. (Simonsen and Rao, *Jour. Chem. Soc.*, p. 232, 1917, record the same m.p.) ; (XI, R=Et), m.p. 133°C. to 134°C. (found : N, 6·6. $C_9H_9O_5N$ requires N, 6·6 per cent) ; and (XI, R=C_4H_9), m.p. 119°C. to 120°C. Finally, the acids were reduced in alcoholic solution with hydrogen under pressure and Raney nickel to the corresponding 4-amino-2-alkoxy-salicylic acids. (XII, R=Me), m.p. 129°C. ; (XII, R=Et), m.p. 143°C. ; and (XII, R=C_4H_9), m.p. 158°C.

Starting from (IX), 5-nitro-2-methylphenoxyacetic acid (XIII), m.p. 175°C. (found : N, 6·4. $C_9H_9O_5N$ requires N, 6·6 per cent) has been prepared and characterized as its ethyl ester (XIV), m.p. 86°C. (found : N, 6·0. $C_{11}H_{13}O_5N$ requires N, 5·9 per cent).

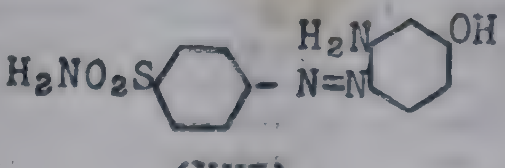


(XIII) (XIV)

Di-azotized PAS has been coupled with β-naphthol and hexylresorcinol to yield the corresponding mono-azo dyes, the dye in the second case being formulated as 4-n-hexyl-6-(3ᴵ-hydroxy-4ᴵ-carboxy) benzene-azore-sorcinol. When di-azotized sulphanil-amide was coupled with PAS in alcoholic sodium-acetate solution, a dye insoluble in sodium bicarbonate solution, and melting at 236°C. was obtained (found : N, 18·4 per cent). The properties of the dye indicated



(XV)

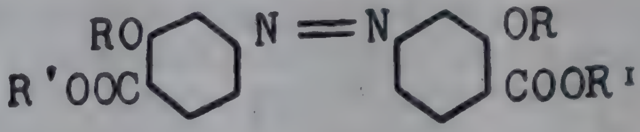


(XVI)

de-carboxylation during coupling, and it was found to be identical with the product of coupling m-amino-phenol with di-azotized sulphanil-amide. Since (XVI) has m.p. 106°C. (IG, *D.R.P.*, **610**, p. 320) the dye from PAS must be constituted as (XV). This unusual de-carboxylation of a salicylic acid derivative by di-azonium coupling is being investigated more closely.

Salts of sulphanil-amides and sulphonic acids have been found to be more active *in vitro* than the parent sulpha drugs by Munshi *et al.* (*Nature*, **161**, p. 55, 1948). Salts of PAS with naphthalene-β-sulphonic acid and with a wetting agent, sodium-di-butyl-naphthalene sulphonate, have now been prepared.

From the azo-salicylic acid (XVII ; R and R'=H), reported last year, the O-diacetyl derivative (XVII ; R=Ac and R'=H) and



(XVII)

the di-n-propyl ester (XVII ; R=H and R'=C_3H_7), m.p. 122°C. (found : N, 7·1. $C_{20}H_{22}O_6N$ requires N, 7·3 per cent), have been prepared.

Many cyanine dyes in concentration as low as 5×10^{-8}M inhibit the oxidative metabolism of *L. Carinii* ('The chemotherapy of filariasis', *Annals, N.Y. Acad. Sci.*, **50**, p. 115, 1948). While amongst the cyanines, 1-ethyl-3 : 6-di-methyl-2-phenyl-4-pyrâmido-2^1-cyanine chloride (No. 863) (XVIII), was found to be one of the most active anti-filarial agents, a comparative study of (XVIII) and a commercial cyanine dye, astraphloxine FF (XIX), used for dyeing textiles, appeared to be of interest. The salts (XX) and (XXI) of (XIX) with potassium guaiacol-4-sulphonate and with sodium-N-lauryl-p-toluidide-2-sulphonate respectively, together with the parent dyes, have been examined by Dr. A. K. Mukerji for their anti-filarial activity. He has found that the anti-filarial activity of (XIX) is almost half that of (XVIII). However, the activity of its salt (XX) slightly exceeds that of (XVIII), while that of the salt (XXI) is more than double that of (XVIII). Toxicity tests have been undertaken.

4. Filaria control unit under the Director, Malaria Institute of India, Delhi.

A. WORK DONE AT DELHI.

I. Major Raghavan who has been deputed from the Malaria Institute of India to supervise the field work of the Filaria control unit at Cuttack prior to starting the field work carried out, in the laboratories of the Institute at Delhi successful experimental transmission of avian malaria—*Plasmodium præcox* of sparrows and reptillian filaria—*C. guindiensis* of *C. versicolor*—in the same specimen of *C. fatigans* Wied. to their respective infective stages—infective larvæ in head/proboscis and sporozoites in salivary glands. There is thus no antagonism between the two for developing in the same mosquito (*C. fatigans*).

II. *Transmission experiments in* S. rhesus *monkeys.*—In the search for a suitable laboratory animal, it was decided to test the suitability of the monkeys. As human material was not easily available at Delhi, it was tried with material from infected *C. versicolor.*

(*a*) Two monkeys were injected with infective larvæ from *C. fatigans* fed on infected calotes. Daily slides were taken from the peripheral blood at 9 p.m., 6 a.m. and 9-10 a.m. One monkey remained negative even till the 22nd week after inoculation, the other died after six weeks of an intercurrent infection. The blood slides were also negative. Post mortem revealed no adult worms in the thigh at the site of the injection, in the abdomen or in the thoracic viscera. Pieces of the viscera have been preserved.

(*b*) Adult male and female worms of *C. guindiensis* were transplanted into the abdomen of monkeys after opening up the abdomen. The monkeys had an uneventful recovery. Blood slides were taken as before, but even after 24 weeks, they remained negative.

As it was felt that the parasite was inhibiting reptiles, there might be a host specificity, it was decided to try injecting infective larvæ of *W. bancrofti* into the monkeys' thighs and study the blood slides. The experiment is still in progress.

B. FIELD WORK AT CUTTACK.

Orissa Province was selected as the venue for trying out the efficacy of insecticidal measures, against the aquatic and adult stages of the mosquito vectors, as compared with the use of drugs on a mass scale, in the prevention of bancroftian filariasis. In consultation with the Director of Health, Orissa, Cuttack was selected as the venue.

A team of workers was organized and deputed under Major Raghavan, Assistant Director, Malaria Institute of India. He arrived at Cuttack on August 11, and within a short time set up a laboratory and an office there. Other facilities, like the hospital and public health laboratory, have also been made available by the kind assistance of the Director of Public Health and other authorities.

Four comparable areas in the outskirts of Cuttack town have been selected. They are all essentials of the urban type as the conditions of overcrowding, ill-developed sanitation and moderately high endemic conditions of filariasis of a typical infected area prevail. Breeding of mosquitoes is extensive. The type of filaria prevalent is *W. bancrofti*, only.

Initial filaria surveys have already been started and positive cases with *microfilariæ* have been selected for treatment with varying dosage regimes. The idea is to work out such a dosage regime as produces a sustained reduction in the numbers of circulating *microfilariæ* to practically *nil* or low level, thus preventing infection in mosquitoes. A free supply of 10,000 tablets of Hetrazan, 50 mg. each, has kindly been arranged by the Secretary, Indian Research Fund Association from the Lederle Laboratories (India).

Propaganda.—The people in these areas are so badly affected that they evince keen interest in any new medicine or cure that they are promised. The nature of our work has been explained to the villagers by talks, collective and individual. The Publicity Department of the Provincial Government has rendered immense help in this work.

List of papers published on filariasis.

BHATE, D. S., PANSE, T. B., and VENKATRAMAN, K. $3 : 3^{1}$-dihydroxy-azobenzene-$4 : 4^{1}$-di-carboxylic acid and 4-amino-salicylic acid (*Proc. Ind. Acad. Sci.*, **29**, p. 196, 1949).

GORE, T. S., PANSE, T. B., and VENKATRAMAN, K. The constitution of citrinin (*Jour. Amer. Chem. Soc.*, **70**, p. 2287, 1948),

GORE, T. S., PANSE, T. B., and VENKATRAMAN, K. Citrinin. Part I (*Proc. Ind. Acad. Sci.*, **29**, p. 289, 1949).

REPORT OF THE FILARIASIS ADVISORY COMMITTEE.

Held in Committee Room 'A', North Block, Central Secretariat, New Delhi at 2-30 p.m., on the 22nd November, 1949.

MEMBERS PRESENT.

Dr. C. G. Pandit (*Chairman*).
Dr. Sangam Lal.
Dr. C. K. Prasada Rao.
Dr. D. N. Roy.
Lieut.-Colonel Jaswant Singh.
Dr. M. O. T. Iyengar (*Secretary*).

Dr. R. G. Dhayagude, Dr. B. B. Dikshit and Dr. A. K. Mukerji were unable to be present.

VISITORS.

Dr. N. V. Bhaduri.
Brevet-Colonel R. N. Chopra.
Lieut.-Colonel A. N. Duggal.
Dr. P. V. Gharpure.
Lieut.-Colonel S. D. S. Greval.
Major S. L. Kalra, I.A.M.C.
Dr. V. R. Khanolkar.
Dr. B. Mukerji.
Dr. J. Mungavin.
Colonel S. Narain.
Dr. (Miss) H. Patil.
Major N. G. S. Raghavan.
Dr. R. Subramaniam.
Dr. K. V. Venkatraman.
Dr. D. K. Viswanathan.
Dr. K. S. Viswanathan.

CONSIDERATION OF THE WORK CARRIED OUT BY INQUIRIES SANCTIONED DURING 1949-50, AND RECOMMENDATIONS IN REGARD TO PROPOSALS FOR 1950-51.

(*a*) *Filariasis inquiry under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta.*

In the absence of Dr. A. K. Mukerji, Dr. N. V. Bhaduri, who was in charge of this inquiry gave an account of the work done during the current year. Screening tests against ***microfilariæ***, of 18 compounds, three received from abroad, and 15 from Dr. K. Venkatraman, had been carried out. For each of the compounds tested, various dilutions were employed with a view to determine the minimal effective concentration, controls being maintained in each set of experiments. It had not been possible to increase the amount of work done because the technique of standardization, trials with various dilutions and selection of suitable material for tests involved considerable time and effort. He stated that he had also planned to carry out intradermal tests with filarial antigen with a view to test the efficacy of drugs but no progress could be made as the antigen was not available. He requested sanction for the continuance of this inquiry during 1950-51 at an estimated cost of Rs. 8,670/-.

Dr. Pandit remarked that the inquiry should be so planned as to provide additional lines of research being carried out simultaneously with the screening tests, in order that the staff under this inquiry may be fully engaged, especially as it seemed likely that the compounds to be tested may not be forthcoming in sufficient numbers. Dr. Bhaduri was, therefore, requested to indicate the additional lines of work which he would be able to take up. Dr. Pandit stated that the proposals put up before the Pharmacology Advisory Committee for a grant to study the pharmacology of the different compounds tested against microfilariæ, had not been recommended by that Committee. In view of the importance of pharmacological studies side by side with screening tests, he felt that it would be advantageous if the two aspects were combined.

The Committee recommended that the Scientific Advisory Board should reconsider the pharmacology proposal and to take it up in conjunction with the continuance of this inquiry and recommended the continuation of the two under a composite unit.

(*b*) *Inquiry to test the usefulness of intradermal and other immunological tests in the diagnosis of early filariasis under the Director, King Institute, Guindy, Madras.*

Dr. Venkatraman gave an account of the work done under this inquiry during the current year. He stated that he proposed to close down the inquiry on 31st March, 1950, and that he was not asking for its continuance during 1950-51. The Committee expressed their appreciation of the excellent work done by this inquiry and agreed to the proposal to wind it up at the close of the current year. In view, however, of the importance of this line of research, the Committee agreed to consider any fresh proposal that might subsequently be put up for an inquiry on the same lines as the existing inquiry or on modified lines.

In view of the persistent demand from various research workers for the antigen which at present is available only from the King Institute, Guindy, Madras, it was suggested by Dr. Khanolkar that other parts of India should be investigated to determine whether there were other foci of *Consipiculum* infection in *Calotes*, so as to increase the availability of the antigen. The Committee endorsed this suggestion and desired that a directive should be issued to research institutes and provincial organizations in the country to carry out a survey of *Calotes* in their respective areas for the occurrence of *Conispiculum* infection.

(*c*) *Inquiry on changing the physical properties of drugs by wetting agents under Dr. B. B. Dikshit, Principal, B. J. Medical College, Poona and Dr. K. Venkatraman, Director of the Technological Department of the University of Bombay.*

The Committee reviewed the note on the work done by this inquiry during the current year. Dr. Pandit spoke on the excellent work carried out under this inquiry. The Committee then agreed to recommend the continuance of this inquiry on the lines suggested and suggested that the officers in-charge of the inquiry may be requested to effect such reduction in the estimates as may be possible.

(*d*) *Filariasis control unit under the Director, Malaria Institute of India, Delhi.*

Colonel Jaswant Singh stated that half of the expenditure on account of this inquiry was being met by the Government of Orissa and that only half of the estimates are debited to the Indian Research Fund Association. In addition to this, the Government of Orissa had provided further facilities and incurred additional expenditure on account of this investigation.

Major Raghavan, who was in charge of the inquiry, stated that the inquiry had been in operation for eight weeks and that much preliminary work had already been carried out. He explained the details and procedure of work.

Dr. Iyengar suggested that the work should be so planned that only one type of control should be employed in any particular experimental area, any one of the three methods, such as (1) anti-larval measures against *Culex fatigans*; (2) anti-mosquito measures, and (3) Hetrazan administration. As the scheme was meant primarily to furnish data on the relative efficacy and relative costs of the different methods of filariasis control, it was essential that there should be no mixing up of the different methods of control in any particular village or unit area.

The Committee recommended the continuance of the inquiry and suggested that Colonel Jaswant Singh be requested to explore ways for effecting economy in cost.

The Committee resolved that the thanks of the Indian Research Fund Association be communicated to the Government of Orissa for their help and co-operation so generously offered at every stage of this research programme.

INDUSTRIAL HEALTH.

1. Industrial health research unit under the Director, All-India Institute of Hygiene & Public Health, Calcutta.

The work of the unit started in January 1948. The unit as a team is doing its work in different fields of industrial health. This year the unit has taken up a few new problems in addition to continuing the work started last year.

1. EFFECTS OF EXCESS NOISE ON WORKERS.

Experts are divided in their views as to whether more or less continuous exposure to noise of about 100 decibels intensity affects the auditory acuity and efficiency of the workers concerned. The two experiments of the unit on this problem will throw more light on the problem as it exists in India.

(*i*) *Effects of excess noise on the auditory acuity.*—A survey of jute-mill workers for the impairment of hearing has been completed. After comparing the data obtained from examination of workers in the most noisy sheds (the spinning and weaving sheds) with those obtained from the control group, it is found that impairment of auditory acuity of the workers in the noisy sheds does not diffier 'significantly' from people working in comparatively quiet sheds.

TABLE I.

Showing the incidence of deafness in the jute-mill workers.

Number of workers examined between 0 to 5 years' service (group A).		Number of workers examined between 5 and above years' service (group B).	Workers with impairment of auditory acuity.	
			Group A.	Group B.
Noisy shed ...	48	57	1	4
Control ..	34	59	1	2

There is no significant increase of deafness in the noisy group.

(*ii*) *Effects of excess noise on efficiency.*—The primary object of the experiment is to determine how and to what extent a reduction in excess noise in jute mills tends to affect the efficiency of the workers concerned. The actual experiment in the Adamjee Jute Mills, Part I of which was reported last year, has been completed. The data obtained are being statistically analysed. The experiment was conducted for 44 weeks but for lack of completeness due to diverse reasons, like shortage of raw jute, 14 weeks' data were discarded. Of those 30 weeks, 15 weeks are with noise (normal condition) and 15 weeks with noise reduced. The different points that are being investigated in Part II of the study are given below :—

(*a*) *Time taken by processes, such as shuttle-loading, shuttle-changing, joining of warp-ends, etc.*—The data for these were obtained, by constant observation with a stop-watch for a period of seven weeks, the performance of eight weavers at their looms. Throughout the working day, the eight weavers were observed by turn for periods of 15 minutes each. The actual time taken by each weaver and by the average weaver has been determined, both with noise and under quieter conditions. Table II gives the group averages, together with the mean variations. Individual data are not being discussed in this short summary:—

TABLE II.

Average time taken for each of the weaving processes in seconds.

Process.	Typical weaver.	Shuttle-loading.	Shuttle-changing.	Joining of broken warp-ends.	
				Simple.	Complex.
Quiet	Mean	13·44	4·27	10·62	17·91
	Mean variations	±2·31	±0·31	±1·25	±2·48
Noise	Mean	13·16	4·30	10·73	17·80
	Mean variation	±2·43	±0·40	±1·26	±2·36

Table III gives the total time which the "average" weaver spends upon each of these processes in every 15 minutes of work. It may be noted that in the 15-minutes work under quiet conditions the average weaver takes six seconds more for joining of complex warp breakages. This six seconds more than outweigh the total benefit of 2·8 seconds in the case of the other three processes derived from a reduction of noise. The interaction of these processes upon each other are being investigated by the statistical method of analysis of variance.

TABLE III.

Total time in seconds, taken by the average weaver for the four processes in 15 minutes' work.

Typical weaver.	Shuttle-loading.	Shuttle-changing.	Joining of broken warp-ends.	
			Simple.	Complex.
Quiet	66·4	20·8	38·6	54·5
Noise	67·8	22·0	38·8	48·5
Difference	—1·4	—1·2	—0·2	+6·0

(*b*) *Various factors and the extent to which they are responsible for the intermittent stoppages of the loom during the working day.*—These factors fall into two groups: 'personal' which includes such factors as going out for tea, water, water-closets, etc., and 'mechanical' which includes all types of mechanical troubles. These data have been obtained for each weaver separately by observing them the whole of the working day for a period of six weeks. Table IV gives the average time lost in the typical day at different hours. This shows the absence of any undue amount of waste of the working time due to causes, personal and mechanical. The loss of efficiency for the average or typical weaver due to these two sets of factors amounts to 5 per cent only.

TABLE IV.

Total time of loom-stoppages for typical day due to personal (P) and mechanical (M) factors in minutes.

Typical weaver	6.30 to 8.00 a.m.	8.0 to 9.0 a.m.	9.0 to 10.0 a.m.	10.0 to 11.0 a.m.	12.30 to 2.0 p.m.	2.0 to 3.0 p.m.	3.0 to 4.0 p.m.	4.0 to 5.0 p.m.	Total
P	4·92	6·79	0·85	0·64	2·75	3·37	1·32	0·26	20·90
M	1·19	0·77	0·44	0·36	1·40	0·67	1·03	0·20	6·06

Average number of stoppages per day { Personal causes 1·78; Mechanical causes 0·52 } On different days they are made at different hours.

(*c*) *Extent to which variation in output is affected by fluctuations in meteorological factors, such as temperature and humidity.*—In other similar studies there has been found some correlation between variation in humidity, for example, and variation in production. The extent of these correlations and their significance, if any, are being determined.

(*d*) *The working efficiency of the individual weavers.*—The variations in efficiency from hour to hour in a typical day and from day to day in a typical week have been worked out, both under noisy and quiet conditions. Analysis of the interaction of these data amongst themselves are being done. Tables of results for this group of problems are not given as the work is not yet completed.

When the various analyses that are in hand are completed, it should be possible to know, apart from the main point, viz. whether noise affects worker's efficiency or not, several other facts of sufficient importance to the jute industry (e.g. the exact time taken for loading a shuttle or the time the worker absents himself from the loom due to personal requirements, etc.), which to our knowledge has not been scientifically investigated as yet in this country.

This study on noise is expected to be completed by December 1949. During 1950 it is proposed to start work on the problem of rest-pauses and hours of work. In most cases in India these are determined from pre-conceived ideas. That a scientific basis for these is essential is evident from experiences of other countries. In munition manufacture in England for example, when hours of work were reduced from 68 to 60 a week, 'not only did the output per hour increase but the total *daily* output increased by about 8 per cent, the worker producing more in 60 hours than he had previously done in 68'. Again 'in an American spinning mill the introduction of two ten-minute rest-pauses a day led to an increase of 13 per cent in production, while labour turnover fell from 25 per cent to practically nil'. Numerous such examples can be quoted. The unit intends, on the basis of the proposed experiments, to suggest to industries concerned the optimum hours of work and rest-pauses. The investigation is complicated and it is hoped that the right amount of co-operation from the factory managements will be forthcoming.

2. INVESTIGATION INTO THE INCIDENCE OF LEAD INTOXICATION IN SELECT INDIAN INDUSTRIES.

The work on lead poisoning was initiated in the printing and soldering industry. Examinations of the workers and the working environment of three of the biggest presses and the soldering department of two industries have been completed. The examination was carried out under the following heads :—

1. Physical examination.
2. Hæmatological examination.
3. Biochemical examination of blood and urine.
4. Industrial air analysis.

The data collected are summarized below and would be ready for publication before the end of the year. The lead-poisoning cases that have been detected have been segregated and the authorities advised on their treatment.

It is proposed to extend the studies in two different types of industries where lead hazard is common : paint industry and foundry.

It is encouraging to note that due to the activity of the unit, some industries with potential lead hazard have become health conscious and the unit is being approached by them for advice. Such advice has been given to the industries involved. These suggestions have improved the working environment in the particular industries as measured by objective tests, for example, by industrial air analysis in industries with lead hazard.

TABLE V.

Showing the incidence of lead hazard in five industries.

Industry.	Number of cases examined.	Mean concentration of lead in mg. per 100 g. blood.	Standard deviation.	Mean concentration of lead in mg. per litre of urine.	Standard deviation.	Mean basophillic aggregation in per cent.	Standard deviation.	Mean concentration of lead in mg. 10 cu.m. air.	Standard deviation.
G.I.P.	40	0·08	±0·06	0·07	±0·04	1·3	±0·54	1·8	±0·36
A.B.B.	31	0·12	±0·03	0·10	±0·05	1·7	±0·45	1·9	±0·20
A.B.E.	20	0·09	±0·06	0·07	±0·05	1·1	±0·40	..	..
M.B.	48	0·08	±0·05	0·10	±0·08	1·2	±0·64	1·7	±0·09
N.C.	52	0·12	±0·04	0·14	±0·04	1·5	±0·21	2·1	±0·09

3. Toxicity of industrial dusts.

With the object of assessing the toxicity, microscopic particles varying from 1μ to 3μ size of the dust were injected in rabbits and guinea-pigs by the intravenous and intraperitoneal routes, respectively. Five kinds of dust (silica, calcite, cement, Kolar gold-bearing dust, Kolar non-gold-bearing dust) have been injected. Post mortem examinations of the injected animals at six-monthly intervals show interesting results, such as progressive fibrotic (cirrhetic) changes in the liver tissue, development of intraperitoneal fibrotic nodules containing a centrally necrosed area. As these tissue changes are progressive and are expected to be maximum and permament after a lapse of two years after injections, final results will be available next year. Table VI gives the number of animals injected and studied so far :—

Table VI.

Indicating the details of animal experiments in progress.

Name of the dust.	Number of guinea-pigs injected.	Number of guinea-pigs on which post mortem done so far for histopathology.	Number of rabbits injected.	Number of rabbits on which post mortem done for histopathology.
1. Silica ..	12	6	6	3
2. Calcite ..	12	6	6	2
3. Cement ..	12	6	5	2
4. Kolar gold-bearing ..	12	6	6	3
5. Kolar non-gold-bearing ..	12	6	..	..
Totals ..	60	30	23	10

It was originally planned to include some more dust in the series, but due to the absence of the Physicist separation of the dust particles of 1μ to 3μ size by 'gravitating separation' method (according to modified Stokes' law) could not be undertaken.

With the object of artificially developing and studying pneumoconiosis in laboratory animals (in the absence of an experimental chamber) rabbits are being kept under observation in the weaving shed of a jute mill. After two years they will be examined for changes in their lungs as a result of continuous exposure to dusts in the working environment of the jute mill. But of the 12 rabbits kept inside the mill, four have died at different periods. The eight surviving rabbits have been under experiment for one year.

4. Relation of environmental warmth to comfort.

This investigation was started in October 1947 with 12 volunteers and was completed in October 1948. The 1,850 comfort votes inside and outside the building have been correlated with readings of a number of meteorological factors. The main results are recorded in Table VII :—

TABLE VII.

Correlation between comfort votes and some essential meteorological factors.

Meteorological factors.	In.	Out.
1. Globe thermometer	0·72	0·84
2. Dry-bulb thermometer	0·74	0·81
3. Wet-bulb thermometer	0·71	0·77
4. Absolute humidity	0·68	0·72
5. Effective temperature	0·75	0·85
6. Modified effective temperature . .	0·74	0·83
7. Mean temperature of the surroundings	0·63	0·81

Further statistical analyses, as multiple correlation, are being carried out with this data.

5. PNEUMOCONIOSIS SURVEY.

The mass chest radiograph unit which has been recommended to the Government by the Indian Research Fund Association Industrial Health Advisory Committee has been sanctioned by Government and it is hoped, will be acquired in the near future. It is proposed to commence in co-operation with the radiographic unit an extensive dust and pneumoconiosis survey in the mines and industries with a pneumocoiniosis hazard. This survey would be the continuation of the preliminary work commenced during the year.

6. SURVEY OF CONDITIONS OF WOMEN IN INDUSTRY.

This survey was contemplated and completed after the last report was submitted. The object of the survey is to determine the actual working and living conditions of women workers in the jute industry. A special questionnaire was prepared and filled up by a Lady Health Visitor after interviewing individually the workers of one jute factory. The most important points covered by the questionnaire pertain to the workers'—

(*i*) Menstrual and pregnancy history.

(*ii*) Service history in the industry with special reference to the exact nature of the work done and habitual postures during the work.

(*iii*) Home conditions with reference to the work done at home and the rest available.

Workers numbering 350 were thus studied and as a control, another 350 non-working women but belonging to workers' families, were further examined. The result will shortly be available for publication.

2. Inquiry into the environmental conditions such as lighting, temperature, humidity, ventilation and dust in factories in and around Calcutta under Professor K. Subrahmanyan at the All-India Institute of Hygiene & Public Health, Calcutta.

The following industries were under investigation :—

Jute mills	2
Printing Presses	2
Flour mills	2
Dhal mills	1
Workshops	2
Glass works	2
Large iron foundry	1

The year brings to an end of two years of work at the jute mills and the printing presses and observation will be discontinued in these in the succeeding year. Observations in the flour mills, dhal mills and workshops were started around June 1948 and will be continued till June 1950. Two glass works and one iron foundry were included in the inquiry during the current year. It is hoped to include one more large iron foundry before the end of October 1949.

Weekly observations on the environmental factors, such as temperature, light intensity, humidity and air movement, are obtained from selected sampling points in each of the above industries. Sampling points are distributed in the factory in a manner to give a representative picture of each place. Alongside the observations taken in each factory, observations on the same environmental factors are also taken for external conditions. Weekly figures on accident, absenteeism and total number of workers in each place were made available for this inquiry. Production figures were available in some industries, while in others it was not possible to gather them. Data collected are plotted on sectional paper to reveal the seasonal variation in these environmental factors and possible correlation between these environmental factors and the accidents, absenteeism or production. In order to reveal the diurnal variation of these environmental factors, observations were taken at intervals of 2 to 3 hours during working hours twice in a year in each of the industries, i.e. once during the summer and again during the winter.

Data collected from the jute mills and printing presses during the first year of the inquiry have been statistically analysed, while the data collected during the second year have not yet been completely analysed. Some of the important findings and conclusions obtained from the satistical analysis of observations taken in jute mills and printing presses during the first year are given below :—

TABLE I.

Maximum, mean and minimum of enviromental factors in the industries.

Industries.	Dry-bulb temperature in °F.			Relative humidity in per cent.			Light intensity in foot candle.			Air velocity in f.p.m.			Effective temperature in °F.		
	Max.	Mean.	Min.	Max.	Mean.	Min.	Max.	Mean.	Min.	Max.	Mean.	Min.	Max.	Mean.	Min.
Jute mills No. 1.	103	87·1	70	88	63	26	80	14	0	140	49·5	5	92·2	81·4	67·8
Jute mills No. 2.	104·5	85·9	75	88	83	26	80	12	0	220	47·4	4	92·2	81·6	66
Printing press No. 1.	99	85·3	71	88	59·5	22	100	12·8	1	430	81·0	9	89.4	79	66
Printing press No. 2.	103	87·6	75	92	60·3	28	200	11·3	0	510	104·6	10	90·4	80	70·3

The average effective temperature, an index of comfort, is not less than 80°F. in three cases, while it was 79°F. in one of the printing presses. The maximum and average dry-bulb and effective temperatures have been recorded highest in the beaming section in both the jute mills and leaving aside the workshop they are found to be the highest in mono-casting department in both the presses. This is due to the presence of hot steam in beaming operation and molten lead in mono-casting operation.

Differences of dry-bulb and effective temperatures recorded inside and outside on any day are also analysed, a summary of which appears below :—

TABLE II.

Difference of dry-bulb and effective temperatures between inside and outside.

Industries.	Jute mills No. 1.			Jute mills No. 2.			Printing press No. 1.			Printing press No. 2		
	Max.	Mean.	Min.	Max.	Mean.	Min.	Max.	Mean.	Min.	Max.	Mean.	Min.
Dry-bulb temperature °F.	11	2·98	0	14·5	3·3	0	13	1·5	0	10·2	1·7	0
Effective temperature °F.	9·8	3·47	0	12·8	4·06	0	6·3	1·4	0	7·2	1·8	0

The inside dry-bulb and effective temperatures were found to be greater than those of outside in most cases for each sampling point. The differences were greater in case of jute mills than those in the case of presses. All the four factories have brick walls but the jute mills have partly corrugated G.I. roofing, while the others have terraced roofing. Comparing these differences in the two jute mills it is seen that these are higher in the second jute mills than in the first one. The walls are of about the same thickness in both the jute mills (the heat transmission coefficient is approximately ·24 BTU per °F.). The floor space in the second jute mills is much more overcrowded than in the first one and each one of the machines and labourers is a source of heat production. This may be causing greater differences between outside and inside temperatures in the second jute mills than in the first one.

Records obtained to show the diurnal variation in these environmental factors were analysed. The dry-bulb temperature inside and outside the industry seem to attain the peak at about the same time. This was found to occur at about 3 p.m. in the afternoon. The differences between the dry-bulb temperature inside and outside at any hour were obtained (Tables III and IV) :—

TABLE III.

Mean difference between inside and outside temperature at any hour of the day in the jute mills.

Department.	Jute mills No. 1.	Jute mills No. 2.
Batching ..	1·61	1·39
Preparing ..	2·51	2·54
Spinning ..	1·51	4·68
Winding ..	3·81	6·62
Beaming ..	3·49	7·6
Weaving ..	2·2	5·29
Finishing ..	1·6	4·49
General ..	1·36	..
Average ..	2·26	4·66

TABLE IV.

Mean difference between inside and outside temperature at any hour of the day in the presses.

Department.	Press No. 1.	Press No. 2.
Melting ..	..	5·8
Linotype ..	1·68	2·37
Ludlow type ..	..	2·37
Mono-perforation ..	2·12	..
Mono-casting ..	3·6	7·37
Composing ..	2·18	0·94
Reading ..	2·51	1·5
Printing ..	1·5	0·94
Hand press No. 1. ..	2·62	..
Hand press No. 2. ..	2·7	..
Envelope-making ..	3·2	..
Counting godown ..	3·37	..
Binding ..	1·76	1·2
Workshop ..	2·31	0·62
Average ..	2·46	2·56

Except for a few departments in all these places this difference of temperature is not very high. The few exceptions are due to presence of hot substances in those parts of the factory.

Weekly accidents, absenteeism, labour strength in each section were made available for this inquiry by both the jute mills. Monthly accident, absenteeism and labour strength were available from the second press, while such figures were unavailable from the other press. Accidents and absenteeism depend on many factors other than the environmental factors under observation. However, comparing these figures for the same department on percentage basis in the jute mills over a period of a year there seems to be a good correlation with dry-bulb and effective temperatures.

TABLE V.

Mean dry-bulb temperature, effective temperature, light intensity and air velocity correlated with accidents and absenteeism.

1. *JUTE MILLS No. 1.*

Period.	Batching						Preparing.					
	D.B.	E.T.	Light	A.V.	% Acdts.	% Abst.	D.B.	E.T.	Light	A.V.	% Acdts.	% Abst.
8-9-47–1-12-47	86·8	79·8	9·6	34·6	0·01	·219	86·9	79·8	8·0	40·4	·007	·103
8-12-47–1-3-48	79·6	68·1	15·7	29·1	0·007	·119	79·4	72·5	14·5	38·2	·002	·134
8-3-48–31-5-48	91·3	84·9	17·3	33·3	0	·276	91·0	83·9	15·3	47·0	·006	·142
7-6-48–30-8-48	90·2	84·9	15·0	37·8	·003	·269	89·6	84·6	17·8	58·0	·002	·421
Year	87·0	79·4	14·4	33·7	·005	·221	86·7	80·2	14·9	45·9	·004	·201
	Spinning.						Winding.					
8-9-49–8-12-47	88·6	82·3	8·8	78·7	·008	·345	89·8	83·1	10·0	41·1	·292	·253
8-12-47–1-3-48	82·8	74·1	11·2	60·0	·006	·303	85·1	76·4	14.3	51·7	·180	·180
8-3-48–31-5-48	93·3	84·8	15·0	65·3	·006	·257	95·6	85·7	20·5	56·1	·293	·257
7-6-48–30-8-48	92·6	86·2	13·5	86·4	·004	·526	94·8	87·5	16.6	69·3	·480	·476
Year	89·3	82·1	12·1	72·6	·006	·357	91·3	83·2	15.3	56·1	·310	·291
	Beaming.						Weaving.					
8-9-47–1-12-47	91·9	84·3	7·2	51·7	·292	·253	89·7	82·9	7·4	45·7	·002	·215
8-12-47–1-3-48	85·5	77·3	8·8	25·9	·180	·180	82·6	74·8	13·8	38·7	·004	·150
8-3-48–31-5-48	96·6	87·0	12·0	34·6	·293	·257	95·0	85·2	16·7	45·9	·010	·253
7-6-48 30-8-48	95·5	87·8	11·1	43·5	·480	·476	92·6	86·3	15·8	52·3	·008	·427
Year	92·4	84·5	9·8	38·7	·310	·291	90·0	82·3	13·4	45·6	·006	·261
	Finishing.						General.					
8-9-47–1-12-47	88·3	81·5	6·7	43·2	·001	·246	86·3	80·3	6·0	61·7	·038	·111
8-12-47–1-3-48	81·7	73·3	13·5	35·4	·001	·188	79·9	72·9	9·8	42·7	·025	·230
8-3-48–31-5-48	92·2	84·4	14·0	23·0	·001	·288	90·9	83·1	11·3	47·6	·040	·261
7-6-48–30-8-48	91·3	85·5	12·1	28·4	·004	·288	89·3	84·3	13·7	47·4	·007	·157
Year	88·3	81·2	11·6	30·0	·002	·237	86·6	80·2	10·4	49·8	·027	·189

TABLE V—(*Contd.*)

II. *JUTE MILLS No. 2.*

Period	Batching.						Preparing.					
	D.B.	E.T.	Light	A.V.	% Acdts.	% Abst.	D.B.	E.T.	Light	A.V.	% Acdts.	% Abst.
10-9-47· 3-12-47	85 2	80·5	12·8	38·3	·054	·035	86·2	78·9	6·8	53·6	·038	·036
10-12-47– 3-3-58	78·2	73·4	11·4	39·7	·050	·030	79·1	75·8	5·5	53·4	·042	·034
10-3-48– 2-6-48	89·6	82·8	24·3	51·3	·069	·100	90·3	84·1	15·0	51·8	·058	·042
9-6-48– 25-8-48	89·4	84·6	26·4	55·8	·032	·054	90·5	84·7	27·3	53·1	·054	·060
Year	85·6	80·3	18·7	46·2	·051	·055	86·6	80·9	13·7	52·9	·048	·043
	Spinning.						Winding.					
10-9-47– 3-12-47	88·1	81·4	6·0	48·7	·035	·110	90·0	82·4	4·5	40·8	·009	·062
10-12-47- 3-3-48	82·5	75·6	7·2	49·8	·030	·071	86·9	77·8	4·4	44·8	·005	·035
10-3-48– 2-6-48	91·0	84·4	8·3	45·1	·021	·069	97·0	86·0	7·6	42·9	·006	·060
2-6-48– 25-8-48	93·6	86·5	15·6	50·8	·036	·081	94·0	87·1	15·6	54·5	·017	·040
Year	88·8	81·9	9·3	48·8	·035	·083	91·9	83·3	8·4	45·7	·009	·049
	Beaming.						Weaving.					
10-9-47– 3-12-47	92·1	81·5	4·4	42·1	·020	·033	88·7	82·2	6·4	34·5	·028	·033
10-12-47– 3-3-48	88·5	77·8	3·5	55·6	·021	·053	86·0	77·4	9·6	37·5	·028	·029
10-3-48– 2-6-48	99·0	86·9	8·45	59·8	·013	·070	95·4	85·2	9·7	50·7	·028	·030
9-6-48– 25-8-48	95·4	88·0	15·9	56·6	·021	·080	93·9	86·8	17·0	61·6	·023	·041
Year	98·4	83·5	8·06	53 5	·018	·059	91·1	82·9	10·7	49·8	·024	·033
	Finishing						Overall					
10-9-47– 3-12-47	85·8	80·4	11·1	32·7	·009	·024	88·0	81·0	7·8	42·2	·027	·047
10-12-47– 3-3-48	82·8	75·6	11·3	35·7	·019	·016	83·1	76·2	7·6	45·7	·027	·028
10-3-48– 2-6-48	94·1	85·2	13·1	30·9	·016	·040	93·5	84·9	12·4	45·7	·030	·060
9-6-48– 25-8-48	91·8	86·3	22·6	50·3	·011	·058	92·7	86·3	20·1	55·8	·027	·060
Year	88·7	81·9	14·5	37·4	·013	·034	90·1	82·1	12·0	47·4	·028	·050

III. *PRESS No. 2.*

Period.	D.B.	E.T.	Light.	A.V.	% Acdts.	% Absts.
5-9-47—28-11-47 ..	86·5	79·9	3·8	77·8	0	38·4
5-12-47—5-3-48 ..	81·3	74·2	4·2	69·2	·003	18·59
12-3-48—4-6-48 ..	92·8	83·3	15·1	131·2	·004	19·46
11-6-48—10-8-48 ..	90·1	83·1	22·2	140·2	·007	15·23
Year	87·6	80·0	11·3	104·6	·003	22·92

Abbreviations.—
D. B. = Dry-bulb temperature in °F.
E. T. = Effective temperature in °F.
Light = Light intensity in foot candle.
A. V. = Air velocity in f.p.m.
% Acdts. = Per cent accidents. % Abst. = Per cent absenteeism.

Light did not seem to have any correlation with these. This is due to the fact that on an average the light intensities at the different sections were quite adequate for the particular types of work carried out in the sections. The accident figures were very low in the press. The thermal environment on the average has been satisfactory and lighting condition was good in the press. Moreover, the machines are fitted with safety devices, the floor space is not overcrowded and the general house keeping was found quite good in the press.

Production figures were available from the second jute mills only. Table VI shows the minimum production has occurred during the period when the effective temperature was highest. Except for one quarter of the year production seems to increase with the decline of effective temperature. Light does not seem to have any correlation with production in the above mills. However, all these conclusions with regard to production are subject to errors due to the influence of other factors such as shortage of raw materials, labour troubles, adjustment of labour strength according to the capacity of the mills with fluctuation of supply of raw materials.

TABLE VI.

Production in relation to environmental factors in jute mills No. 2.

	Dry-bulb temp. in °F.			Light intensity in foot candle			Air movement f.p.m.			Effective temperature, °F.			Production in tons per capita per day.
	Max.	Mean.	Min.	Max.	Mean.	Min.	Max.	Mean.	Min.	Max.	Mean.	Min.	
Sept., Oct. and Nov.	98	88	80	29	7·8	1	135	42·2	5	89·2	81·0	74·2	·0236
Dec., Jany. and Feb.	95	83·1	70	28	7·6	1	120	45·7	8	83·1	76·2	67·8	·0235
March, Apr. and May	104·5	93·5	79	60	12·4	0	130	45·7	6	92·2	84·9	71·5	·0236
June, July and Aug.	102	92·7	84·5	80	20·1	1	148	55·8	4	91·6	86·3	67·8	·0215
Average	104·5	90·1	70	80	12	0	148	47·4	4	92·2	82·1	67·8	·0230

It has been proved by the other workers in the field that production rate declines very rapidly as effective temperature exceeds 80°F. This, however, could not be verified in this investigation for want of controlled conditions to compare with. The findings of this inquiry seem to indicate that an effective temperature of 85°F. may be the limit of appreciable fall in production in jute mills for the clothing that jute-mill workers in this area wear. Average effective temperature in both the jute mills had been above 80°F. and in one of the presses it was 80°F., while in the other it was found to be 79°F. (*vide* Table I). This shows that the thermal environment in the jute mills should at least be improved to give a higher *per capita* production.

Data collected from other industries are also being statistically analysed on the lines adopted for the jute mills and the presses.

3. Inquiry into the problem of traffic accidents and accident-prone personnel amongst bus and tram drivers in Calcutta under Mr. S. K. Bose at the University College of Science & Technology, Calcutta.

In addition to the 140 drivers tested in 1948-49, 153 more bus and tram drivers have been tested.

Some changes in the tests and testing procedures have been made. The Triangular Form Board used last year has been replaced by a type of Nail Board. In the steadiness test the presentation time has been increased to 10 seconds. Previously it was five seconds.

A modified form of dotting test has been used. The modification was suggested by Professor G. Bose (now retired). In its present form the test is designed to measure the capacity for sustained attention, quick response and discrimination. The test is indicative of temperamental qualities also. Three varieties of geometrical figures, circles, squares and triangles, are printed on an endless paper-belt which is made to glide over a rotating drum controlled electrically for an optimum speed. A wooded horizontal platform over the belt has a small slit cut on it so that figures on the moving paper belt are exposed. The subject has to put dots with a sharp pencil on all the circles through the slit. The scores of this test have given the best correlation with numbers of accidents made by the drivers in their first six months' service. The merit of the test will be proved if about one hundred accident records which will be available by the end of the current year, i.e. December, correlate highly with the scores made by the persons concerned on this test.

The reaction time test is being modified by varying the position of the light stimulus from experiment to experiment so that the subject will have to be on the alert to look for it anywhere within a specified area.

In the administration of the battery of tests and in working out the statistical calculations some of the post-graduate students of the Department of Psychology of the Calcutta University have rendered valuable service in scientific interest only.

(For the purpose of preparing a battery of tests for detecting driver-trainees who will be susceptible to accidents it is not necessary to test more tramway personnel. Only the accident records of those tested will have to be examined in relation to their test scores. A few may have to be re-tested. By arrangement with the Directorate of Transport of West Bengal a number of bus driver-trainees will be tested from December. By the end of May 1950, it is expected that the requisite number of bus drivers will be tested. After that the data collected will have to be statistically treated and the follow-up records of the drivers to be examined. Another year will be needed to complete the inquiry.)

The estimate of the trainers of the driver-training schools is being correlated with the final grading made on the basis of the tests. Positive and significant correlation has been obtained so far. In case of the tram driver-trainees those who can finish their training early are allowed to leave the Training School and drive cars. Thus, the period of every trainee's stay in the Training School indicates his capacity for quick learning. The final test grading will be correlated with the period of stay.

In correlating test scores with accidents a practical difficulty is experienced. The accidents as recorded by the Transport Companies or by the Police are not always due to the fault of the drivers. It is hard to find out in every case of accident whether it is caused by the fault of the driver or by that of others who gave the driver concerned no chance to save the situation. This factor may be responsible for rather low correlation between final gradings and accident records of some individuals. Such cases are being re-examined.

Steps have been taken to contact Professor Eric Farmer of the U.K. for his advice.

The total number of 153 persons tested in the current financial year so far have been classified according to their age groups, educational level and previous occupation.

Age in years.	Number.	Education.	Number.
20 to 24 ..	116	Illiterate ..	25
25 to 29 ..	30	Class I to III	31
30 to 34 ..	3	Class IV to VI	67
35 to 39 and above	4	Class VII to IX	22
		Class X and above	8
Total ..	153		153

Previous occupation	Number.
Unemployed	36
Casual labourer (coolie)	22
Cultivator	11
Darwan, peon, etc.	50
Shopkeepers, mill workers, primary school teachers, etc.	34
	153

On the basis of the tests the persons tested were graded :—

Final grading	Number	Remarks
A (superior) ..	*Nil*	
B (above average) ..	27	Should do well.
C (average) ..	100	May develop and do.
D (below average) ..	24	Very doubtful.
E (inferior) ..	2	Will not do.

The correlations of the final gradings with the trainers' estimates and accident records in the first six months are approximately 0·7 and 0·5, respectively. The accident records of only about 75 persons have been available.

4. Inquiry into sickness absenteeism due to accidents under the Chief Medical Officer, Bata Shoe Factory, Batanagar.

The objects with which the inquiry was undertaken were to :—

(*a*) Study all available accident records with a view to analyse the causes of accidents, the agency causing accidents, disability and absenteeism in relation to the age, occupation and other factors personal to the worker.

(*b*) Calculate the inquiry frequency and severity rates.

(*c*) Calculation of absenteeism, disabling injuries and disability.

Study of accident records.—Accident records numbering 15,228 from 1943 to 1948 were analysed and are shown in Table I :—

TABLE I.

Total number of accidents from 1943-48.

Accidents.	1943	1944	1945	1946	1947	1948
Reportable ..	747	784	429	907	792	1,252
Non-reportable	1,766	1,480	2,666	1,792	1,868	785
Totals ..	2,513	2,264	3,095	2,659	2,660	2,037
Number of factory working days		307	254	266	206	252
Accidents per day		7·38	12·2	9·995	12·9	8·07

The study reveals that only about 38 per cent of the accidents are due to machinery, the remainder 60 per cent being due to the personal factors of the worker. Rubber, leather and machinery departments contribute to the largest number of accidents. There are more accidents on the first working day of the week (Monday) than on the last working day (Friday). The accident curve shows a double rise during the period of 2 to 2½ hours after starting the work. The night-shift accidents are comparatively small most probably due to better and uniform illumination.

Lacerated injuries, contusions and abrasions account for the greater number of disabling injuries. The parts of the body affected most are hands, fingers and feet or toes.

The cost of absenteeism worked out to Rs. 16 to Rs. 27 during 1945-47. Annual absence in days per 1,000 workers per annum is shown in Table II :—

TABLE II.

Annual absence in days per 1,000 workers per annum.

Year.	Working staff.	Absent, days.	Absence in days per 1,000 workers.
1945 (May to Dec.)	254,917	3,962	15·54
1946 ..	303,324	17,558	57·50
1947 ..	241,448	10,235·5	42·37
1948 ..	337,211	17,643	52·31

Study of accidents.—Accidents numbering 686 occurred during the period August 1948 to March 1949. Of the total number 531 (77·41 per cent) were reportable and 155 (22·59 per cent) non-reportable. Exhaustive study of these accidents individually shows that :—

(1) Only 34·8 per cent of the accidents are associated with machines ; remainder 65·2 per cent being due to non-machinery agencies.

(2) Unsafe working conditions accounted for 128 accidents out of the total number of 686.

(3) The unsafe acts of the worker accounted for 317 accidents, while unsafe personal factors in the worker accounted for 329 accidents.

(4) Departments contributing to the largest number of accidents are machinery repair, rubber and leather cutting, and shoe and cutting departments.

(5) Temperature at which accidents occur varies between dry-bulb 68°F. to 92°F., wet-bulb 58°F. to 84°F., globe 74°F. to 96°F.

(6) Illumination responsible varies from 0·2 to 0·8 candle power.

(7) The workers concerned have either little previous training or dissatisfaction with the present sickness benefit scheme.

(8) Environmental and socio-economic factors which may have a bearing in the cases studied are bad housing, anxiety due to indebtedness and long separation from families for want of accommodation.

(9) Only 20 per cent of the workers studied have satisfactory nutrition.

Recommendations.—Since the maximum number of accidents occurs during the third hour of the shift, it is suggested that a 10 to 15 minutes recess after about two and half hours' work instead of five hours' continuous work would offer mental and physical relief to the worker and would go a long way in refreshing him and reducing the number of accidents.

5. Inquiry into the prevalence of byssinosis in cotton textile industry in South India under Dr. T. S. Adisubramaniam, Municipal Health Officer, Mathurai.

This inquiry has been in progress for the last two months of August and September 1949. During this period of two months, 250 workers in the Madura Mills were clinically examined and the results of the investigation are submitted below :—

The lines of work undertaken are :—

(1) Study of old records of sickness absenteeism.

(2) Study of dust-producing conditions in the various departments.

(3) Clinical examination of the workers in the mixing, blowing, and card-rooms.

(4) Study of blood picture of the workers.

In this investigation four schedules have been put into operation and data are being collected for final analysis. These schedules are :—

Schedule I : Information regarding the individual worker.
,, II : Information regarding personal data, previous history, education, etc.
,, III : Information regarding the workers' opinion of his job.
,, IV : Information regarding the worker and his social and home environments.

1. Study of old records of sickness absenteeism.

The sickness absenteeism statements of the Madura Mills due to diseases for the last three years (1946, 1947 and 1948) reveal that respiratory diseases have been steadily increasing, as the following Table will indicate :—

Year :—	1946.	1947.	1948.
Total working staff of workers ..	1,32,559	1,33,991	1,35,686
Total number of sickness treated ..	26,775	28,445	34,187
Total number of respiratory diseases other than pneumonia and T.B. treated	2,630	3,000	4,037
Percentage of total sickness to total working staff	20·18	21·22	25·20
Percentage of respiratory diseases to total number of sickness treated ..	9·826	10·58	11·80
Percentage of respiratory diseases to total working staff	1·985	2·246	2·975

The various lung infections are detailed below :—

Diseases.	Total number treated.		
Influenza	1,119	1,898	1,419
Pneumonia	110	86	55
T.B.	29	16	16
Respiratory diseases* other than pneumonia and T.B.	2,630	3,009	4,037
Total cases treated	26,775	28,445	34,187
Total accidents	721	722	714

---

*The data re. the various kinds of respiratory diseases (grouped under this head) are not available.

The number of man-days lost and the cost of sickness absenteeism due to sickness are tabulated below :—

Year :—	1946.			1947.			1948.		
Number of man-days lost*	146,273			143,034			139,072		
	Rs.	as.	p.	Rs.	as.	p.	Rs.	as.	p.
(*a*) Sick-leave wages paid	50,835	13	0	54,901	15	6	62,821	12	6
(*b*) Hospitalization costs	3,890	1	0	7,790	6	9	9,394	8	3
Total costs (*a* and *b*) ..	54,725	14	0	62,692	6	3	72,216	4	9
Total accident-compensation paid ..	6,050	6	9	5,638	8	3	6,976	7	6

*One " Man-day " means eight working hours per day.

2. STUDY OF DUST PRODUCING CONDITIONS IN THE MADURA MILLS.

The first process the raw cotton undergoes is the mixing of one staple with another. Much dust and dirt is disengaged in this operation. The respiration is affected from the dust irritating the respiratory passages. The arms and hands show dry skin, and often a goose-skin appearance. In two cases, (dry) eczema has been observed. After passing through the mixing-hoppers, the cotton passes through the openers and scutchers. The strippers, grinders, and card-room workers are engaged in the next process of cotton-yarn production. They often suffer from dry cough, sore-throat and with occasional tightness of chest. The next place, where the cotton comes to, is the drawing and roving rooms. The ring frames are the next, where the cotton assumes the cotton-thread form. High temperature is present here for two reasons, viz. :—

(*i*) The machines are working at high speed.
(*ii*) The machines are situated in the ground floor with not good ventilation.

The next process is packing where there is little dust. Workers look very healthy here.

Within the mills the dust is disturbed by the following factors, viz. :—

(*i*) Moving bales on the road from the stock piles—sand and cotton dust.
(*ii*) Throwing bales on the road with a thud after transporting them.
(*iii*) Breaking the bales in the mixing room—coarse and fine cotton dust, as well as sand dust.
(*iv*) Feeding the hoppers by hand—coarse cotton fibres set afloat.
(*v*) In the stock-bins for collecting cotton from the crigthon openers—coarse cotton fibres float freely here. Thread-extractors operate here.
(*vi*) Hopper openers—fine dust float,

(*viii*) In the card-room—fine cotton dust floats, giving a cloudy appearance to the whole area, in spite of being cleaned by vacuum pump thrice daily.

(*ix*) At the willow machines—cotton, sand and husk dusts are set afloat.

(*x*) Due to the high speed of the roving frames—coarse and fine cotton fibres fly off in very large numbers.

(*xi*) House-keeping by constant sweeping of the floors is a factor, in the disturbance of dust, which rises from the floor and irritates the nostrils and throat.

From the high velocity of the machines in use, the quantity of short fibres of cotton set afloat in the mixing and blow-rooms is very great indeed. The speed of the beater, scutcher and of the fan are as follows :—

Speed of:	Revolutions per minute.
(*i*) Line shaft	300
(*ii*) Beater (for coarse counts)	1,000
(*iii*) Beater (for fine counts)	857
(*iv*) Finisher—beater (course counts) ..	857
(*v*) Finisher—beater (fine counts) ..	923
(*vi*) Fan (coarse counts)	1,160
(*vii*) Fan (fine counts)	996
(*viii*) Finisher fan (fine counts)	1,057
(*ix*) Finisher fan (coarse counts)	995

Dust control and removal.

Except in the 'combing' section there is no sprinkler installation. The rubber department is air-conditioned.

Lighter dusts are removed in the mixing and blow-rooms by means of the exhaust fans, which take them to the gutter and flue. Cards are stripped by vacuum-stripping process, reducing the brush-stripping by hand to the minimum. This eliminates the floating cotton and metallic dusts as far as possible. House-keeping system is adopted which removes the coarse cotton dust and waste.

3. CLINICAL EXAMINATION OF 250 WORKERS IN THE MIXING, BLOW, AND CARD-ROOMS.

The workers engaged in the mixing and blow-rooms were selected to ascertain the extent to which health is affected by the cotton fibres and dust, as these are the two dusty sections of the cotton-yarn mill. Of the 250 workers who have been examined, 40 per cent show symptoms of respiratory affection, while the remaining 60 per cent are free from any respiratory disease.

The clinical diagnoses of 250 workers are as follows :—

	Number.
Respiratory diseases	80
Heart diseases (high blood-pressure and V.D.H.)	15
Other diseases	15
With no symptoms	140
Total ..	250

The respiratory diseases from the point of view of cotton dust factor are classified as :—

Room.	Byssinosis.	Bronchitis.	Asthma.	Allergy.	T. B.	Total affected.	Total non-affected.	Total examined.
						Per cent.	Per cent.	
Mixing— ..	16	12	4	4	..	36 (30)	84 (70)	120
Blow— ..	8	4	..	4	4	20 (40)	28 (60)	48
Card— ..	..	..	4	4	..	8 (25)	24 (75)	38
Strippers ..	..	..	18	18	..	36 (72)	14 (28)	50
Totals ..	24	16	26	30	4	100 (40)	150 (60)	250

It may be pointed out that the strippers suffer most, while next come the blow-room workers, and then the mixing and card-rooms workers, in the descending order of frequency.

Symptoms of byssinosis.

Even though the classical symptoms and signs of *pucca* cases of byssinosis have not been detected, the *observed* signs and symptoms suffice to diagnose the nature of disease.

After working for a few years (minimum eight years in this Madura Mills) without any appreciable trouble or complaint, the worker develops dry cough and attacks of breathlessness, with or without a feeling of tightness in the chest. The dry cough which at times is distressing (as it is accompanied by a feeling of tightness in the chest and breathlessness or tiresomeness) is relieved by going on sick leave for two to three days. On return to the working spot and after three to four days they exhibit the same above symptoms of cough and breathlessness. This is most frequently observed in the mixing and blow-rooms. The workers lose flesh and become thin, both in face and body, even though they have not developed the typical *facies byssinosis*. Evidence for the *mill fever* is slender and, therefore, not considered at present.

Cases on the border-line between byssinosis, asthma, allergy and eosinophil lungs have been noted, but the numbers are not large enough to evaluate the data so far collected.

There are some workers who in the early months of their career in the mills showed allergic reactions (sneezing, cold, cough with breathlessness) to cotton dusts ; but subsequently have become immunized so to say, as they have been working without any complaint for the rest of their service. The service of the 250 workers (under review) ranges from 23 to 27 years.

4. STUDY OF THE BLOOD PICTURE OF THE WORKERS.

A systematic and routine examination of blood for total r.b.c. and w.b.c. counts, differential counts, and hæmoglobin estimation have been carried out. The results are given below :—

Total numbers examined :—	Affected group.	Non-affected group.	Grand total.
	90	60	150
Averages.—			
R.b.c. counts per c.cm. ..	2,788,750	3,815,000	
Leucocyte	8,533	5,591	
Ratio of w.b.c. to r.b.c. ..	1 : 326·8	1 : 682·3	
Changes in r.b.c. :—			
Microcytic changes, per cent	75	*Nil*	
Differential count :—			
Polymorphos, per cent ..	61·6	73	
Lymphocytes per cent ..	24·4	15·0	
Large mononuclears per cent	6·4	7·0	
Eosinophils per cent ..	7·6	5·0	
Percentage of hæmoglobin * ..	55·0	80·0	

* Estimated by Sahli-Adams haemometer.

The reduction in the number of r.b.c. and the hæmoglobin percentage with marked microcytic changes point to a high rate of anæmia among the affected workers. The other set of 60 non-affected workers, who are also working at the same spot in the mixing and blow-rooms, also show a reduction of r.b.c. but without any changes in the r.b.c.

The high percentage of eosinophil cells in both sets of workers reveal that inhalation of cotton dust over a long period tends to produce eosinophilia.

Further study is required to confirm this blood picture to correlate the data of the changes in the blood picture.

The assessment of the state of nutrition among this group of 150 workers shows that the sub-nutritional group is highest.

State of nutrition.	Affected group.	Non-affected group.
Excellent	7	15
Normal	25	30
Sub-normal	43	10
Bad	15	5
Total ..	90	60

This assessment of the state of nutrition has been made according to the recommendations of the Board of Education, England, as applied to school children. From careful observations, it may be pointed out that the poor state of health is due to : (*i*) Bad food-habits and (*ii*) economic conditions, where the workers with a large family is unable to feed himself properly. Data regarding nutritional survey are being collected.

A general survey of social and environmental conditions have been carried out. This survey reveals that most of the workers are indebted, as they are not able to meet their family budgets within their average monthly income of Rs. 74/-. The high cost of living index is one of the main reasons for this state of affairs. As the quantity of rationed rice is not enough, rice in the black-market is bought at a high price.

For lack of the necessary instruments, dust counts and meteorological reading could not be carried out at present. Arrangements are being made to take skiagrams of affected workers to aid the clinical diagnosis. Biological investigations could not be carried out for lack of facilities. The results of these proposed experiments will be reported later on.

REPORT OF THE INDUSTRIAL HEALTH ADVISORY COMMITTEE.

Held in Committee Room 'B', North Block, Central Secretariat, New Delhi at 10 a.m. on the 24th November, 1949.

MEMBERS PRESENT.

1. Lieut.-Colonel C. K. Lakshmanan (*Chairman*).
2. Dr. T. S. Adisubramaniam.
3. Dr. A. C. Banerjea.
4. Mr. N. S. Mankiker.
5. Dr. (Miss) S. Pandit.
6. Dr. K. Rajagopal.
7. Dr. L. M. Sen.
8. Professor K. Subrahmanyan.
9. Dr. M. N. Rao (*Secretary*).

Major V. M. Albuquerque was unable to attend.

VISITORS.

1. Group-Captain D. N. Chakravarty.
2. Dr. T. Chandrasekharaiya.
3. Dr. M. B. Daver.
4. Dr. S. S. De.
5. Lieut.-Colonel A. N. Duggal.
6. Lieut.-Colonel S. D. S. Greval.
7. Dr. M. N. Gupta.
8. Dr. Gwash Lal.
9. Mr. Jaleshwar Prasad.
10. Dr. C. L. Katial.
11. Dr. Muktha Sen.
12. Lieut.-Colonel D. P. Nath.
13. Dr. V. V. Puri.
14. Dr. R. Subramaniam.

The Chairman welcomed the members and visitors who were present at the meeting. He then referred to the financial position of the Indian Research Fund Association and informed the Committee that as a result of the recent economy measures adopted by the Government of India, the grant-in-aid to the Association was also likely to be cut down to a considerable extent. He, therefore, impressed on the members the necessity of a very careful scrutiny of each and every proposal and suggested that only those inquiries which were considered to be of an urgent nature should be recommended.

The Chairman then reminded the members that the country was rapidly getting industrialized and that the problems of industrial health, therefore, were becoming more numerous. With the industrialists becoming more conscious of the health of the industrial labour, an urgent need had been created to carry out active research in the various problems affecting the health of the labourers. While expressing disappointment at the absence of any new proposal which perhaps was due to the dearth of trained personnel in this particular field, he stressed the importance of carrying out researches into the more urgent problems with the limited facilities and workers available in this country and requested the members to help in the formulation of lines for future research.

Item 1.—TO CONSIDER THE WORK CARRIED OUT BY THE INQUIRIES SANCTIONED DURING THE YEAR 1949-50 AND TO MAKE RECOMMENDATIONS REGARDING THE LINES OF WORK FOR 1950-51.

(*a*) *Industrial health research unit at the All-India Institute of Hygiene & Public Health, Calcutta.*—The Chairman mentioned that the industrial health research unit was just completing two years of its activities. He then outlined the work so far done on this unit. The Co-Officer in-Charge of the unit then acquainted the Committee with the detailed programme of the unit and especially to the work nearly completed, full details of which were presented to the members in advance :—

(*i*) The investigations on the incidence of lead poisoning in printing presses and soldering operations indicated the following :—

(*a*) There was no indication of any acute cases of lead poisoning in any of the two hundred operatives investigated upon.

(*b*) A fair percentage (30 to 40 per cent) of these cases showed a pathological accumulation of lead in their systems as judged by the amount of lead in blood and urine and the basophilic aggregation of red blood corpuscles.

(*c*) The symptomatology and signs in these sub-clinical cases of lead poisoning are not consistent with the accepted symptomatology in acute cases.

During the discussion on this piece of investigation a suggestion was made that these symptoms should be taken as a " red signal " for the impending development of the disease in an acute form. The Committee agreed with this suggestion. The Committee laid stress on the importance of taking adequate measures for the rehabilitation of cases of lead poisoning occurring in printing presses and allied industries. It was further recommended that in addition to the investigation being carried out in printing presses, a few more industries where lead was being used to a greater extent, viz. electrical accumulator works, paint industries, etc., should be thoroughly investigated to find out the incidence of acute lead poisoning cases also. It was felt that in these industries the number of cases of acute lead poisoning would be considerable.

(*ii*) The second investigation on the effect of excess noise on the efficiency of workers in the jute textile industry was reported to be very nearly completed. The effects of excess noise on the auditory acuity and the efficiency of jute weavers was explained and discussed.

(*iii*) Other activities of the industrial health research unit included study of toxicity of industrial dusts, relation of environmental warmth to comfort, study of the conditions of women in industries, etc. These investigations were reported to be still in progress.

A suggestion was made that future researches should be so planned as to be of immediate use in the present-day problems of industrial health. It was proposed to commence investigations on the problem of rest pauses and hours of work. It was felt that on the basis of this investigation, the Committee might be able to make some definite recommendations on the subject.

After a full discussion on the various investigations undertaken by the unit and the proposed programme of work for the next year, the Committee unanimously recommended the continuance of the industrial health research unit.

(*b*) *Inquiry into environmental conditions such as lighting, temperature, humidity, ventilation and dust in certain industries in and around Calcutta under Professor K. Subrahmanyan at the All-India Institute of Hygiene & Public Health, Calcutta.*—The Officer-in-Charge of the inquiry explained the lines of investigations undertaken during the year and also the proposed lines of work for the next financial year. It was also explained that by the end of the next financial year enough data would be available to enable him to give some concrete recommendations in regard to environmental conditions and that this inquiry was more of a fact-finding nature, on the findings of which a broader scheme of research could be planned by others. There was an active discussion on the various facts presented by the inquiry and it was generally agreed that no definite conclusions could be reached by the investigation at this stage. A suggestion was made that this inquiry could be made more useful to the industries concerned by considering the facts obtained in the light of the manufacturing process requirements of the industries.

The continuation of the inquiry was recommended with the budget as proposed.

(*c*) *Inquiry into the problems of traffic accidents and accident-prone personnel amongst bus and tram drivers in Calcutta under Mr. S. K. Bose at the University College of Science & Technology, Calcutta.*—The Committee noted that the work of the inquiry was making good progress and that it was to be completed during the next financial year. The data collected during the current year had to be unified and analysed before a full report on the work done by the inquiry could be prepared. The Committee noted that only part-time assistance of two psychologists had been asked for and recommended that the inquiry be continued during the next financial year with the budget as proposed.

(*d*) *Inquiry into sickness absenteeism due to accidents at the Bata Shoe Factory under the Chief Medical Officer, Batanagar.*—It was noted that the inquiry had terminated with effect from the 30th June, 1949. The Chairman placed before the Committee a complete report on the work done on the inquiry which was received by him for publication from the Assistant Research Officer lately employed on the inquiry. It was emphasized that the published reports on inquiries recommended by the Committee should be authoritative as these recommendations would have to be adopted in future planning. After considering the report, it was decided that it should be scrutinized carefully by the Chairman before publication. The Committee further recommended that the permission of the Bata Shoe Company should be obtained before action was taken to publish the report.

(*e*) *Inquiry into the prevalence of byssinosis in the cotton textile industry, in South India under Dr. T. S. Adisubramaniam, Municipal Health Officer, Mathurai.*—The Officer-in-Charge of the inquiry explained to the Committee that the inquiry was in progress for just over three months and that the work done so far could not, therefore, lead to any definite conclusions. While scrutinizing the report, the Committee felt that it covered a rather wide range of subjects, such as social aspects of the workers, their environments, sickness absenteeism in the factory and routine examinations of the blood, etc. It was felt that these aspects need not be investigated into but that the inquiry should restrict itself specifically to the terms of reference namely, the presence or absence of byssinosis in the cotton textile industry in India as could be judged by the complete investigations in one of the Madura mills. The Committee, therefore, recommended that the Officer-in-Charge of the inquiry should restrict himself to this aspect of the work only and all other studies, such as physical environment of the worker regarding dust, heat, humidity, etc., should not form part of the inquiry. Subject to this restricted programme the continuation of the inquiry was recommended. The provision made in the budget for the post of research assistant was deleted at the suggestion of the Officer-in-Charge of the inquiry.

Item 2.—TO NOTE THE COMMENTS OF DR. T. BEDFORD ON THE REPORT OF THE 4TH MEETING OF THE INDUSTRIAL HEALTH ADVISORY COMMITTEE HELD IN NEW DELHI ON THE 10TH DECEMBER, 1947.

Noted.

Item 3.—OTHER BUSINESS.

Mr. Mankiker, the Chief Adviser of Factories, brought to the notice of the Committee certain difficulties which the Factory Inspectorate were confronted with, especially in regard to the suitability of certain roofing materials for the factories in the tropics. He stated that the present knowledge on the subject was far from adequate and suggested that investigations on the subject should, if possible, be undertaken. The Committee after some discussion on the subject decided that the industrial health research unit should, with the limited means at its disposal, carry out investigations on the subject in co-operation with the Chief Adviser of Factories. Mr. Mankiker assured the Committee of full co-operation in the investigation of problems on industrial health which may be undertaken by the industrial health research unit. The Chairman, on behalf of the Committee, thanked Mr. Mankiker for his offer of help and hoped that this co-operation will prove of great benefit in the investigations undertaken by the unit.

Group-Captain Chakravarti brought to the notice of the Committee certain problems which the Royal Indian Air Force was faced with. He especially wanted the advice of the Committee in regard to an investigation into the problem of noise created while testing jet engines of the aeroplanes used by the Air Force and also on other problems of Aviation Medicine, viz. clothing for bomber pilots, measurement of fatigue in aviation personnel, comfort in the cockpit, etc. The Committee suggested that these problems should, as far as possible, be investigated into with the active co-operation of the industrial health research unit. The Secretary of the Committee was requested to go into the details of the investigation and explore the possibilities of making use of the personnel of the industrial health research unit to a limited extent for this purpose.

Dr. A. C. Banerjea, Director of Medical and Health Services, United Provinces, brought to the notice of the Committee the acute dearth of medical personnel trained in industrial hygiene. He stressed that unless early steps were taken to institute some sort of training in this field it would be absolutely impossible to tackle the present-day problems of industrial health. The Committee unanimously agreed that with the industrial expansion now taking place in the country, it was most essential to have adequate trained personnel in the field of industrial hygiene if health standards were to be maintained in the mills, factories and mines in the country. The Chairman informed the Committee that proposals for instituting at the All-India Institute of Hygiene & Public Health, Calcutta, a course of training extending over a period of one year, leading to the degree of 'Diploma in Industrial Health' had already been submitted by him to the Government. The Committee felt that this course should be instituted without any delay and recommended that the Scientific Advisory Board of the Indian Research Fund Association be requested to bring this recommendation of the Committee to the notice of the Government for very early action.

Dr. Banerjea also brought to the notice of the Committee the problem created by the pollution of water sources by sugar and shellac factories in the United Provinces by discharge of the industrial wastes without treatment into rivers. He urged the Committee to consider ways and means of preventing such pollution which had become an urgent public health problem in his province. Professor Subrahmanyan acquainted the Committee in detail about the work that was being done at the All-India Institute of Hygiene & Public Health, Calcutta on the subject. Industrial wastes from more than fifty representative factories in and around Calcutta had been studied and a stretch of Hooghly river into which these industrial wastes were being discharged, surveyed with a view to assess the problem of river pollution by industrial wastes. He also gave his experiences of the problem in strawboard and other factories in India. The Committee, after discussion on the subject, agreed that Dr. Banerjea should, in co-operation with the All-India Institute of Hygiene & Public Health, Calcutta, start preliminary investigations with pilot disposal plants. Dr. Banerjea assured the Committee that he would be glad to give all facilities for such investigations to be undertaken and would also be able to meet such incidental expenses in this connection as may be necessary.

The Chairman in his concluding remarks thanked the members for the valuable suggestions made at the meeting.

RABIES.

REPORT OF THE RABIES ADVISORY COMMITTEE.

Held in the Central Secretariat, North Block, New Delhi at 10 a.m. on the 21st November, 1949.

MEMBERS PRESENT.

Lieut.-Colonel M. L. Ahuja. (*Chairman*).
Dr. C. B. D'Silva.
Lieut.-Colonel S. D. S. Greval.
Dr. R. Sanjiva Rao.
Dr. K. V. Venkatraman.
Dr. N. Veeraraghavan.
Dr. A. G. Brooks. (*Secretary*).

Dr. S. R. Pandit was unable to be present.

VISITORS.

Dr. H. K. Dutta.
Dr. S. S. Pillai.

1. The proposal for a rabies inquiry under the Director, Central Research Institute, Kasauli was considered essential and the Committee recommended that a grant of Rs. 5,150 be sanctioned for the inquiry.

2. The extract from the minutes of the 9th meeting of the Medical Research Liaison Committee held in India House, London, on the 2nd March, 1948 was considered. It was resolved that Professor Harington be thanked for his suggestion, but in view of the fact that the 'culture' vaccine is already under investigation at Kasauli and as no definite results have yet been obtained, it was not considered necessary at the present stage to send material for examination to other countries.

3. The question of the establishment of the rabies research centre at Kasauli was considered. The Chairman informed the Committee that the matter was discussed at the last meeting of the Pasteur Association of India in May, 1949 and it was decided that the estate ' Shelton Lodge ' adjacent to the Central Research Institute, Kasauli be purchased for the proposed centre, by direct negotiation between the Secretary of the Pasteur Association and the owner of ' Shelton Lodge '. Owing to the increase in cost of building material, the Association also agreed to defray expenses up to a total of Rs. 2,00,000 this sum represented additional grant of Rs. 50,000 over and above the original amount of Rs. 1,50,000 already promised in 1946. As it is now three years since the proposal was first mooted, the Rabies Advisory Committee recommended that the Pasteur Association of India be requested to take steps for the immediate acquisition of ' Shelton Lodge ' to enable the proposed rabies research centre to commence functioning at an early date.

4. The report of research work on rabies carried out at the Central Research Institute, Kasauli, which had previously been circulated, was discussed. It was resolved that the report be incorporated as an *Appendix* to this Report.

5. Lieut.-Colonel S. D. S. Greval reported that he was carrying out certain serological investigations which involved the use of time-expired antirabic vaccine as antigen in complement-fixation tests. It was decided that samples of such vaccines possibly lying with workers in the various institutes be sent to Colonel Greval for investigation. It was also decided that: *(a)* All institutes engaged in the preparation of antirabic vaccines in India should send specimens of their rabies fixed virus to the Central Research Institute, Kasauli for titration, and *(b)* the virus now in process of fixation by Dr. Veeraraghavan should be sent to all antirabic institutes in India, to be used as a test virus in experimental work, and for the purpose of periodical checking of vaccines in routine use at all Centres.

APPENDIX.

Report of the research work on rabies carried out at the Central Research Institute, Kasauli, during the year 1949.

At the last meeting of the Rabies Advisory Committee, held at Madras on 30th November, 1948, a preliminary report of work carried out at the Central Research Institute, Kasauli was submitted. The report suggested that 'culture' vaccine as prepared by Dr. Veeraraghavan at Coonoor was possibly superior in antigenic value to the Semple 5 per cent vaccine in general use throughout India. The present report concerns further experiments carried out in this connection during the current year.

1. It was claimed that the live virus in the culture medium was killed by 0·5 per cent phenol in 72 hours. The criterion for death of the virus accepted by Dr. Veeraraghavan was failure to infect guinea-pigs when the phenolized medium was injected subdurally into them. We have found that 0·5 per cent phenol does not kill the virus in a 5 per cent brain emulsion in 72 hours and that mice can easily be infected with this material. Again using a 3·3 per cent brain emulsion, as is present in the 'culture' medium it was found that 2 out of 4 mice died of rabies when injected subdurally with the phenolized 'culture' medium after it had been incubated for 72 hours, whereas guinea-pigs injected subdurally with the same inoculum survived. The fact that the 'culture' vaccine contains live virus probably accounts for the good results we have obtained with the vaccine, in experiments both at Kasauli and Coonoor, as we have invariably used freshly prepared 'culture' vaccine. As it contains live virus it is possible that the vaccine will not prove entirely free from risk and the life of the vaccine may be very limited.*

2. It was claimed by Dr. Veeraraghavan that the virus multiplied, on incubation, in a medium consisting of sheep brain extract, glycine, peptone, normal horse serum and distilled water to which an inoculum of fresh fixed virus was added. The inoculum is heavy so that the 'culture' medium contains 3·3 per cent of fixed virus to commence with. Accordingly, the titre of the virus contained in the medium was tested before and after incubation. The first sample tested had a titre of 10^{11} which fell to 10^{10} after 24 hours incubation. The second sample fell from 10^{12} to 10^{9} after incubation.

3. It was thought possible that some ingredient in the medium injured or irritated brain tissue when injected subdurally into animals and that this resulted in making a weaker infecting dose effective. Accordingly, a fixed virus brain emulsion was prepared and mixed with various ingredients as shown below and titres tested, without incubation.

Titre of virus.	10^3	10^4	10^5	10^6	10^7
Virus in distilled water only ...	2/2	2/2	2/2	2/2	0/2
Virus in distilled water *plus* peptones ...	2/2	2/2	2/2	2/2	0/2
Virus in distilled water *plus* glycine ...	2/2	2/2	2/2	2/2	2/2
Virus in distilled water *plus* glycine and peptones	2/2	2/2	2/2	2/2	2/2

Numerator = deaths. Denominator = total number used.

***N. B.—Dr. Veeraraghavan, however, informed the Committee that he used old vaccines in his experiments and that these vaccines were incubated for six days prior to use.**

Comment.—There was thus no increase in virus content in the medium when incubated, on the contrary there was a decrease. The subdural injection of glycine would appear to render guinea-pigs more susceptible to fixed virus injection. Unfortunately no end point was reached in animals given glycine. The experiment is to be repeated.

4. It is generally believed that the whole brain emulsion as used in Semple's vaccine is superior in immunizing efficiency to the supernatant fluid of the vaccine from which most of the brain tissue has been removed. Experiments conducted at this Institute indicate that this is a misconception and that in reality there is little difference in titre and in antigenic response between the supernatant fluid and the whole brain emulsion. Further experiments in this connection are in progress and encouraging results with the vaccine prepared from the supernatant fluid have been obtained.

5. As reported last year the brain of a sheep which died of fixed virus infection has been stored in 50 per cent neutral glycerin at 0°C. and tested at intervals. The titre of this brain has remained fairly constant when tested, as shown in Table I :—

TABLE I.

Date of test.	Age of virus, (months).	$\frac{1}{250,000}$	$\frac{1}{2,500,000}$	$\frac{1}{25,000,000}$
7-6-1948	1	2/2	1/2	1/2
8-7-1948	2	2/2	0/2	0/2
10-8-1948	3	2/2	2/2	0/2
10-9-1948	4	2/2	2/2	0/2
8-10-1948	5	2/2	1/2	0/2
12-11-1948	6	2/2	2/2	...
7-1-1949	8	2/2	2/2	...
8-3-1949	10	2/2	2/2	0/2
6-5-1949	12	2/2	1/2	...
8-7-1949	14	2/2	1/2	...
9-9-1949	16	2/2	2/2	...

Comment.—After an initial fall the titre has remained more or less constant under the conditions described.

6. Many workers, particularly in the early days of rabies research following Pasteur, reported the presence of virus in various tissues. A goat with street virus was infected and attempts were made to recover virus from the various tissues from 15th day after infection and daily thereafter. The goat developed rabies on the 19th day and died two days later. Guinea-pigs were used as test animals in this experiment. Results are shown in Table II :—

TABLE II.

Inoculum.	Days after infection.	Route of infection.	Quantity injected, c. c.	Date of infection.	Date of 1st symptoms.	Date of death.	Remarks.
Milk ..	15	Subdural	0·2	17-4-49	..	..	Lived 3 months
„ ..	16	„	„	18-4-49	..	..	„
„ ..	17	„	„	19-4-49	..	20-4-49	Brain injury
„ ..	17	I.M.	1·0	19-4-49	..	..	Lived
„ ..	18	S.D.	0·2	20-4-49	..	..	„
„ ..	18	I.M.	1·0	20-4-49	..	..	„
„ ..	19	S.D.	0·2	21-4-49	..	..	„
„ ..	19	I.M.	1·0	21-4-49	..	..	„
„ ..	19	I.M.	1·0	21-4-49	..	..	„
„ ..	20	S.D.	0·2	22-4-49	..	..	„
„ ..	20	I.M.	1·0	22-4-49	..	..	„
„ ..	20	I.M.	1·0	22-4-49	..	..	„
„ ..	21	S.D.	0·2	23-4-49	..	..	„
„ ..	21	I.M.	1·0	23-4-49	..	..	„
„ ..	21	I.M.	1·0	23-4-49	..	..	„
Saliva ..	20	I.M.	2·0	22-4-49	12-5-49	13-5-49	N.B.†
„ ..	21	I.M.	2·0	23-4-49	13-6-49	13-6-49	N.B.†
„ ..	21	I.M.	2·0	23-4-49	31-5-49	1-6-49	N.B.†
„ ..	21	I.M.	2·0	23-4-49	..	..	Lived 3 months
Blood ..	21	I.M.	1·0	23-4-49	..	..	Lived
„ ..	21	I.M.	1·0	23-4-49	..	..	„
Muscle in saline ..	21	I.M.	2	23-4-49	..	..	„
„ ..	21	I.M.	2	23-4-49	..	..	„
Urine ..	19	I.M.	2	21-4-49	..	..	„
„ ..	20	I.M.	2	22-4-49	..	..	„
Brain ..	21	S.D.	0·2	23-4-49	3-5-49	5-5-49	N.B.†
„ ..	21	I.M.	2·0	23-4-49	3-5-49	5-5-49	N.B.†

Comment.—The quanitty of the virus in the milk and muscle of this goat was so little, if present at all, as to be negligible and it was not possible to infect guiena-pigs with these materials, even when they were injected subdurally. The experiment is of practical importance as numerous inquiries are received regarding the possible danger of drinking milk of an animal which has developed rabies. The experiments are to be continued and if, as seems likely, it is found that milk consistently fails to transmit rabies it will be justifiable to refuse treatment to those now treated for the supposed risk they run.

Attempts were made last year to grow rabies street virus in the living tissues of various yeasts and fungi. These failed. This year we have tried to cultivate the virus in the growing tissues of plants such as tomato and pumpkin. The raw growing vegetables were inoculated with an emulsion of street virus and then allowed to ripen. When fully mature the pulp of the vegetable, which showed necrosed areas at the site of injection and elsewhere, was injected into guinea-pigs. Agar tubes inoculated with material from the necrotic areas, in the case of the pumpkin, remained sterile. The inoculated animals have remained free from disease.

PHARMACOLOGY.

1. Inquiry on indigenous drugs under Dr. J. C. Gupta at the School of Tropical Medicine, Calcutta.

The indigenous drugs inquiry, one of the most important and useful branches of investigation in India, has reached its second year of existence since the break in 1946. Although the work has been very much handicapped from the very beginning owing to difficulties, in the procurement of suitable and genuine drugs for various reasons, fairly good progress has been made in the line. Full stress on the detailed pharmacological examination with the isolated total and individual active principles of the drugs has been given throughout the investigations. Three original articles have recently been sent to the press, out of which two papers have already been accepted for publication in the *Indian Journal of Medical Research.* Two other papers are expected to be ready for the press shortly.

I. *Dæmia extensa* Linn.—A paper on the pharmacological action of *Dæmia extensa* Linn. *vis-á-vis* posterior pituitary extract has been sent for publication. As already remarked in the earlier reports, it has important functions on the uterus. In many respects it behaves like ergot and pituitrin. The following are some of the important findings of the investigations :—

1. *Dæmia* and pituitrin appear to differ in their site of action on the uterus.

2. Unlike pituitrin, progesterone does not appear to inhibit the action of *Dæmia* on the uterus.

3. *Dæmia* appears to have a beneficial effect on the atonic intestine of paralytic ileus.

4. A state of mild hyperglycæmia which disappears quickly, appears to result from the administration of *Dæmia* ; a mild hypoglycæmia seems to develop later whose significance has not yet been studied.

5. The administration of *Dæmia* appears to increase the total acid and diminish the free acid of the gastric juice, particularly after a milk feed.

Clinical trials are undergoing at different hospitals and the report so far obtained reveal its unfailing usefulness in conditions like menorrhagia, metrorrhagia and sub-involution of the uterus. Administered after the third stage of labour, it appears to complete normal involutions. Reports regarding its utility in post-partum hæmorrhage are still awaiting. Attempts are also being made to find out if its various pharmacological actions could be accentuated or improved by substances acting synergestically or antagonistically, as required, with the active principles of the drug. In fact. problems of interest are cropping up in divergent lines in connection with the drug.

II. *Cissampelos pareira* Linn.—This drug contains about 1 per cent of total alkaloids and the pharmacological studies reveal that :—

(*a*) The alkaloids tend to lower the blood pressure.
(*b*) They tend to increase the intratracheal pressure.
(*c*) Atropine appears to influence the fall of blood-pressure and the

rise of intra-tracheal pressure, thereby tending to attribute a cholinergic character to the drug.

(*d*) The movements of the isolated intestine of cat appears to be depressed after an initial stimulation.

(*e*) The movements of the isolated uterus of the cat does not appear to be influenced appreciably.

Further pharmacological investigations are being continued.

III. *Fractionation of Cissampelos pareira* Linn.—The alcoholic extract was evaporated to dryness, dissolved in water and fractionated with the help of different solvants. Each of the ether sulphuric, chloroform and ethyl acetate fractions was found to contain alkaloids, the proportion of alkaloid, soluble in ether sulphuric, being less than that of the chloroform-soluble one. The chloroform-soluble one, however, was soluble in water in greater proportion. Besides these the residual aqueous portion gave tests for carbohydrate, indicating thereby the probable presence of substances like glycosides. An alkaloid from this portion has also been separated which on pharmacological examination appeared to be weakly cholinergic in character causing a slight depression of blood-pressure and slight increase of the intra-tracheal pressure, both these effects disappearing after atropine. Detailed pharmacological investigations will be persued.

IV. *Dregia volubilis*.—A preliminary examination regarding the water soluble active principles from *Dregia volubilis* showed that it appeared to have the effect of increasing the systemic blood-pressure in cat after a preliminary fall. Further purification of the active principle which is glycosidic in nature has been done and the pharmacological examinations so far made indicate that it is perhaps primarily cholinergic in character and to some extent directly stimulant to involuntary muscles as well. Further elucidation regarding its pharmacological character and that of resin is in progress.

Alkaloids have also been detected in the drug but owing to their presence in minute quantities it has not yet been ascertained whether further work with this fraction will be worth undertaking.

V. *Rauwolfia total alkaloids*.—A method has been devised for the estimation of *Rauwolfia* total alkaloids in urine. It appears that there is some relationship between the urinary excretion of total alkaloids and their hypotensive effect. During the lowering of blood-pressure the urinary elimination of the alkaloids was low, while when the blood-pressure was not affected, about 50 to 60 per cent of the alkaloids was eliminated through the kidney within 4 hours of the intake of the drug. Further work on this line for fuller elucidation on the subject with special reference to the individual alkaloids will be taken up in the near future when the fractionation of the *Rauwolfia* total alkaloids which is in progress will be complete.

A paper has already been sent to the press and has been accepted for publication in *Indian Journal of Medical Research*.

VI. *Rauwolfia resins*.—Several trials have been given with an injectible form of *Rauwolfia* resin on rats to study its sedative properties. The results are not yet conclusive. Further work in the line is under progress and the result will be communicated in due course.

VII. *Vitex peduncularis.*—The isolation of the active principles of the drug has not yet been possible and efforts are being made in various lines to isolate the same. The work is under progress.

VIII. *Michelia champaka* Linn.—An alkaloid has been isolated from the bark which has been subjected to pharmacological examinations but its exact nature has not yet been ascertained. A few bitter principles of the drug have also been separated and the pharmacological investigations of those and of the alkaloid are in progress.

IX. *Scindapsus officinalis* Schott Melet.—The pharmacological investigations with the drug gave indications of the presence of a principle slightly stimulating the blood-pressure. Further extensive pharmacological studies on the drug are being continued.

X. *Holarrhena antidysenterica* Wall.—Recent studies with the plant, Kurchi (*Holarrhena antidysenterica* Wall) revealed that the presence of the active principles depended not only on the age of the plant and the season of collection but there was a distinct difference in the distribution of the alkaloids in the various parts of the plant. Thus, the total alkaloids in the bark and root-bark were greater in the months of February and August (average 3·7 per cent) than in April and May when their contents fell to a minimum (2·9 per cent in the case of root-barks and 3·3 to 3·1 per cent in the case of barks). In stem, the alkaloid content was highest in January (0·57 per cent), July (0·569 per cent) and August (0·568 per cent) and minimum in February (0·256 per cent). The total alkaloids in leaves were comparatively less in February and March (average 0·58 per cent), they then gradually increased from April attaining the maximum value in the month of June (1·55 per cent), while in July and August there was again a fall and the contents came down to a value as low as a little over 1 per cent. From these it is also obvious that most of the alkaloids are present in the barks and root-barks. Considerable variation of the alkaloids in the bark due to age of the plant was also noticed and the following will give a more exact picture of the changes :—

Age of the plant in years.	Percentage of total alkaloids in the bark.
5	2·303
8	3·390
12	3·372
20	2·834
25	3·061

These observations suggest that in order to get the maximum yield of the active principles the appropriate portion or portions of the plant should be collected at the right age and proper season. This opens up a new line and it is highly probable that such investigations in connection with all the drug of therapeutic reputation will be fruitful and have commercial importance.

In order to study the influence of soil, age and season on the accumulation of active principles in different parts of the plant it is necessary to have plantation of the herbs under expert supervision wherefrom the drugs of varying ages could regularly be supplied for examination at different seasons.

It will be really helpful if the agricultural farms at different places under the State control, take up the question and start cultivation of the drugs with a view to study the effect of soil, season and age on the accumulation of active principles in medicinal plants.

2. Inquiry on indigenous drugs under Brevet-Colonel R. N. Chopra at the Drug Research Laboratory, Jammu.

Chemical and pharmacological studies of medicinal plants (*Sarcococca purniformis* Lindl. (vern. *Sangli.*), is an evergreen glabrous shrub which grows wild in temperate Himalayas from Murree to Bhutan, in Khasia and Manipur ranges in Assam and in the Deccan peninsula on the Western Ghats, from Canara southwards generally at altitudes from 4,000 feet above sea-level. It grows abundantly in Kashmir and is extensively used by the local people as a febrifuge and in the treatment of rheumatic affections.

As a result of analysis of the leaves a crystalline alkaloid with m.p. at 236°C. to 238°C. a crystalline neutral product m.p. 251°C. to 252°C. and an orange-colouring matter have been isolated. The base gave hydrochloride salt m.p. 268°C. to 270°C. (decomp.), hydroiodide m.p. 242°C. to 243°C., chloroplatinate m.p. 212°C., auric chloride 197°C. (decom.), methiodide m.p. 274°C.

Pharmacology.—One per cent solution of the total alkaloids was used in the preliminary investigation of its pharmacological action.

The alkaloid is toxic to the *paramæcia*, 1/1,000 concentrations of the alkaloid killing this protozoa in 4 to 5 minutes. It has, however, no bactericidal action.

When administered to dogs under anæsthesia, the alkaloid produces some fall in blood-pressure which does not come to its original level. The heart rate remains unaltered but there may be distinct decrease in the force and amplitude of the ventricular systole.

The respiration also showed slight slowing. The involuntary muscles were practically unaffected. Further studies will be continued.

Ajuga bracteosa Wall (vern. *Jan-i-adam*) is a small herbacious plant growing in the Himalayas at altitudes of 2,000 to 3,000 feet above sea-level. In Kashmir it grows commonly up to an altitude of 2,000 feet in Drawa, Bhadrwa and Riasi. It is extensively used in the indigenous medicine as a bitter, astringent and in the treatment of febrile conditions. It is also used by the villagers as blood purifier and to kill lice. As a result of preliminary work on the whole plant a non-glucosidic bitter substance and potassium nitrate have been found to be present. Further work is being continued.

Substitutes for pharmacopeial drugs.—*Ergot* has been reported on grasses in Simla (Himachal Pradesh) and in several places in South India. Investigation on these grasses were started with a view to see if the naturally growing ergot is active. Samples of ergots found on the grasses *Brychopodium sylvaticum* Beauv., *Oplimanus compositus* Beauv., and on *Chrysopogon* species of grasses were procured from Simla. The collection was done in the month of November and the ergots on these grasses were well matured. The natural ergots gave no colour with para-di-methyl-amino-benzol-dilyde solution showing the complete absence of the ergot alkaloids. This confirms Padwick's work who reported that ergot found on grasses in Simla is *Claviceps virdis* and is different from the official fungus *Claviceps purpures*. It appears that *Claviceps virdis* does not develop the active alkaloids of ergot. Further specimens are being examined to confirm this work.

Male fern. Dryopteris blanfordii (Hope) C. Ch. is a beautiful shuttlecock fern which grows in abundance in *Pinus excelsa* forests. Samples of this fern obtained from Kalatope and Chhatri (near Chamba) on analysis have shown the complete absence of calcium oxalate crystals, percentage of total ash varies from 3·1 to 3·6, percentage of acid-insoluble ash from 0·4 to 4·3, percentage of fillcin from 3·5 to 4·1 and extractive with ether from 8·2 to 8·5 per cent. The fern comes up to the official standards and can substitute the official drug as an athelmintic against tapeworms.

Benzoin.—Styrax benzoin Dyrand which yields the gum benzoin does not grow in India but there are several other species of *styrax*, i.e. *S. hookeri* Clarke (*lepcha-chamoking*) and *S. sarrulatum* (*lepcha-chamo*) which grow wild in Sikkim and Bhutan. In order to study if the gum resins from these species can be used in place of gum benzoin, the Forest Officer of the Sikkim State was requested for the supply of the gum resins who reported that his several attempts to tap this gum from a number of these trees were unsuccessful and he could not obtain any appreciable quantity of the gum. It would appear that these species cannot be utilized for the production of gum benzoin.

Styrax benzoin tree has been raised in the botanic garden, Bangalore. In order to study the quality of the gum, the superintendent of the garden was requested for the supply of the gum who has reported that several of his attempts to tap the gum were unsuccessful. It appears, therefore, that the large scale propagation of this tree cannot be undertaken in Mysore State.

Adulteration of drugs used in the indigenous system of medicine.—Vaidyas, Hakims and manufacturing firms dealing with the manufacture of preparations on Ayurvedic and Unani medicine in this country complain that they often do not get genuine drugs and those obtained in the market are invariably adulterated. With a view to standardize these drugs, a large number of samples is being examined so that some definite standards can be established. There has been a demand all over the country for establishment of such standards and it is hoped that gradually all commonly used drugs will be tested for their potency and standardization.

The following drugs were examined during the year :—

1. Aconite roots were found to be mixture of *Aconitum chesmanthum*, *A. plamatum*, *A. lacinatum*, *A. balferi* and the alkaloidal content varied from 0·8 to 4·79 per cent.

2. *Extract berberis* (Vern. *Rasaut*) : Samples analysed have shown the berbarine content to vary from traces to 18·2 per cent and the ash was as high as 49·1 per cent. None of the samples came up to official standards. The chief adulterant detected was dried molasses (*gurh*).

3. *Saffron :* Large number of the samples on analysis showed that only two samples were genuine and the other were parts of flowers dyed with organic dyes.

4. *Gum mastic :* Analysis of the samples showed acid number varying from 60·1 to 156·1. Only three samples gave acid number near about 60 (that of gum mastic), the rest gave very high acid number showing calophony as the main adulterant.

3. Inquiry on the action of snake venoms and their constituents on the biochemical activities of tissue-cells under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta.

1. *Effect of banded krait venom on the anærobic oxidation of different metabolites by tissue-cells.*—In the previous reports, it has been recorded that the ærobic oxidation of substrates like glucose, sodium lactate, sodium succinate, p-phenylene-di-amine, etc., by pigeon's brain tissue is markedly inhibited by minute doses of the banded krait venom and from this it has been inferred that the venom contains an inhibitor of the cytochrome-cytochrome-oxidase system. In this connection, it is considered necessary to investigate whether the other respiratory enzymes, such as lactic dehydrogenase, succinin dehydrogenase, pyruvic dehydrogenase, etc. are also affected by krait venom. This has been done by carrying out the oxidation in the anærobic phase by Quastel's ferricyanide technique (*Biochem. Jour.*, **32**, p. 936, 1938).

Technique : Immediately after killing the pigeon, its brain was taken out, cooled on ice for 15 minutes and chopped and minced to fine paste. One hundred mg. of this paste were weighed out accurately in separate Warburg's flasks and suspended and broken up in bicarbonate-Ringer solution prepared according to Quastel (*loc. cit.*). The venom dissolved in bicarbonate-Ringer solution, was added in adequate amount to the system where necessary. 0·2 c.c. of ferricyanide-bicarbonate solution (prepared by mixing 5 c.c. of 10 per cent $K_3Fe(CN)_6$ with 1 c.c. of 0·16 M $NaHCO_3$) together with 0·1 c.c. of 0·1 per cent methylene blue in water were also added to the brain suspension. Suitable controls were kept without venom. 0·5 c.c. of a 1·2 per cent solution of the substrate in bicarbonate-Ringer was kept in the side tubes of some of the flasks, whilst 0·5 c.c. of bicarbonate-Ringer was kept in the side tubes of the others to serve as control. The total volume of liquid in each flask was 3·0 c.c. The flasks were fitted to the manometers and the air was displaced by a mixture of 95 per cent N_2 and 5 per cent CO_2. To ensure perfect anærobiosis, small pieces of yellow phosphorus were kept in the inner cup of each of the flasks. The flasks were shaken for 1 hour and 30 minutes in a thermostat-bath at 37·5°±0·1°C. By this process, sufficient time was allowed to the venom to produce its effect and moreover, the brain tissue was depleted of a part of the substrates already present in it. The substrate solution was then tipped off from the side tubes into the reaction mixture, the level of liquid was adjusted in the manometers and the evolution of CO_2 was measured for 1 hour at intervals of 15 minutes. Some of the results are recorded in Table I :—

TABLE I.

Substrate used.	Mg. venom per c.c. of brain suspension.	Percentage inhibition of oxidation of substrate.	Mean percentage inhibition.
Sodium lactate (0·2 per cent)	0·00067 ..	55 46 44	49
	0·00130 ..	71 75	73
Sodium succinate (0·2 per cent)	0·00330 .	7 0 0	2
	0·0067 ..	0 0	0
Sodium pyruvate (0·2 per cent)	0·00067 ..	31 26	29
	0·00130 ..	56 57 52	55

From the results recorded in Table I, it will be evident that the banded krait venom has very little action on the anærobic oxidation of sodium succinate ; thus a dose of 0·0067 mg/c.c. of the venom which produces an inhibition of 75 per cent during the ærobic oxidation of succinate, has no effect on the anærobic oxidation of the same substrate. This fact lends support to the view that the observed effect is due to the inhibition of the cytochrome-oxidase system. The anærobic oxidation of the sodium lactate and sodium pyruvate are, however, strongly affected by the venom. As the oxidation of the last two substrates takes place through the mediation of co-enzyme I, it is not possible to say whether the inhibition of oxidation is due to the action of the venom on the protein moiety of the dehydrogenases, or on the carriers, viz. co-enzyme I and cocarboxylase. It may be mentioned in this connection that Chain (*Biochem. Jour.*, **33**, p. 407, 1939) suggested that the inhibition of the oxidation of substrates requiring co-enzyme I by the snake venoms is probably due to the breakdown of co-enzyme I by enzymes called nucleotidase contained in the venom. Experiments were, therefore, carried out to determine the effect of banded krait venom on the anærobic oxidation of sodium lactate by pigeon's brain tissue in presence of added co-enzyme I.

The technique of experiment was the same as described previously with this modification that after incubating the brain suspension with the banded krait venom for 1 hour and 30 minutes, the substrate together with co-enzyme I was tipped off from the side tube into the main compartment, and the evolution of CO_2 was determined for 1 hour at intervals of 15 minutes.

The results indicated that even in the presence of 1·0 mg. of co-enzyme I, the inhibition of the anærobic oxidation of lactate by a dose of 0·00067 mg. of banded krait venom per c.c. of the brain suspension was about 20 per cent, the corresponding figure in the absence of added co-enzyme I being about 50 per cent. The failure of the added co-enzyme I to completely eliminate the inhibition by the venom may be due either to the splitting of the co-enzymase by the venom, or to the presence of another inhibitor in the venom. The elucidation of this point was sought by studying the effect of heat (*i*) on the nucleotidase activity and (*ii*) on the oxidation-inhibiting capacity of the venom.

To study the effect of heat on the activity of the nucleotidase in the venom, 0·5 per cent solutions of the krait venom in distilled water were heated at different temperatures for 30 minutes. The solutions were then cooled, and 0·2 c.c. of each were added to bicarbonate-Ringer, pH 7·4, contained in the main compartment of the Warburg's flask, while 1·0 c.c. of co-enzyme I in bicarbonate-Ringer (2 mg./c.c.) was kept in the side tubes of the flasks. Suitable controls were also kept. The flasks were fitted to the manometers and the air was displaced by a mixture of 95 per cent N_2 and 5 per cent CO_2. The entire arrangement was shaken in a thermostat-bath at 37·5° ± 0·1°C. for a period of 15 minutes. The co-enzyme I was tipped into the main compartment after this period and the rate of breakdown of co-enzyme I was studied by estimating the CO_2 evolved at intervals of 10 minutes. From the results, it was found that the nucleotidase in the krait venom was completely inactivated by heating the venom at 63°C. for 30 minutes.

The effect of banded krait venom heated at 63°C. for 30 minutes to destroy the nucleotidase, on the anærobic oxidation of sodium lactate by pigeon's brain tissue was then studied. The experimental technique was the same as described previously. It was found that the venom heated at 63°C. for 30 minutes does not lose its property of inhibiting the oxidation of lactate, although it loses its nucleotidase activity completely. This suggests the presence of another inhibitor in krait venom, quite distinct from the nucleotidase present in it. Whether it produces the inhibition of the lactic acid oxidation by acting on the lactic dehydrogenase, or on some point of the co-enzyme I molecule other than that affected by nucleotidase remains yet to be investigated.

Further experiments on the effect of heated krait venom on the oxidation of substrated, such as malic acid, glutamic acid, etc., which also require co-enzyme I for their oxidation, are necessary. It is also intended to take up the problem of isolation of this inhibitor of anærobic oxidation.

2. *Specificity of the inhibitor of the cytochrome-oxidase system isolated from cobra venom.*—In the previous report, evidence was given regarding the presence of an inhibitor of the cytochrome-oxidase system in cobra venom and a method for its separation in a purified state from the other active principles in the crude venom, was developed. In banded krait venom, the presence of an inhibitor of the cytochrome-oxidase system has also been detected. As both the venoms, i.e. cobra and banded krait, contain the same active principle, it was considered desirable to investigate the specificity of the inhibitors by immunological tests.

The inhibitor of the cytochrome-oxidase system was isolated from the venom of the cobra by the method described in last year's report. The m.l.d. of the inhibitor for a rabbit was determined by intramuscular injection and found to be 0·2 mg. A rabbit was immunized against this inhibitor by a series of injections thrice a week over a period of three months. After this period was over, the rabbit was bled and the serum separated from the blood clot.

One c.c. of the venom solution (either cobra or banded krait) was mixed with 0·5 c.c. of the antiserum and the mixture was kept at 37·5°C. for one hour. The solution was then centrifuged and the number of units of the inhibitor in the supernatant solution was determined by studying the effect of the solution on the oxygen uptake of pigeon's brain in presence of p-phenylene-di-amine. A parallel experiment with normal rabbit serum was also carried out to serve as control. A unit was defined as that amount of the venom, which, when allowed to act on 50 mg. of freshly chopped and minced pigeon's brain tissue for 1 hour and 30 minutes in presence of air, would subsequently inhibit the oxidation of 0·167 per cent p-phenylene-di-amine by 45 to 50 per cent in one hour. The results are shown in the Table II :—

TABLE II.

Type of venom.	Number of units of inhibitor in the venom used.	Amount of antiserum added, c.c.	Number of units of inhibitor after the reaction.	Number of units of inhibitor neutralized.
Cobra venom ..	6·00	0·5	1·00	5·00
	4·00	0·5	0·00	4·00
	2·00	0·5	0·00	2·00
Banded krait venom	2·00	0·5	1·00	1·00
	1·25	0·5	0·00	1·25

The results indicate that the antiserum, while neutralizing 4 units of cobra venom completely cannot neutralize even 2 units of banded krait venom. The results show the difference in the immunological behaviour of the inhibitor present in the venoms of the cobra and the banded krait.

Further experiments with the sera prepared against the inhibitor in krait venom are in progress.

3. *Effect of snake venoms or tissue glycolysis.*—It is a well-known fact that in addition to the energy derived from the process of oxidation in the tissues, a part of the energy is also derived from the glycolysis taking place in them. It has been reported already that cobra venom can inhibit the process of glycolysis to a marked extent in minute doses. Experiments are being carried out to separate this inhibitor of glycolysis from cobra venom and to study its mechanism of action.

INDIAN RESEARCH FUND ASSOCIATION.

REPORT OF THE SECOND MEETING OF THE PHARMACOLOGY ADVISORY COMMITTEE HELD IN THE CENTRAL SECRETARIAT, NORTH BLOCK, NEW DELHI AT 2-30 P.M. ON THE 21st NOVEMBER, 1949.

MEMBERS PRESENT.

Brevet-Colonel Ram Nath Chopra. (*Chairman*).
Dr. B. N. Ghosh.
Dr. J. C. Gupta.
Dr. B. Mukerji.
Mr. P. M. Nabar. (*Secretary*).

VISITORS.

Group-Captain D. N. Chakravarti.
Major S. L. Kalra.
Colonel S. Narain.
Dr. C. K. Prasada Rao.
Dr. D. N. Roy.

The Chairman in his opening speech welcomed the members and stated that, while considering the various schemes put forward before the Committee, they should take into account the existing economic stringency. The grant-in-aid to the Indian Research Fund Association might be reduced by the Government of India and a corresponding reduction will have to be made in the various proposals. He stated that the question as to what reduction could be effected in the existing schemes and whether the new inquiries proposed in the agenda should be postponed will have to be carefully examined.

Item 1.—To consider work done by the existing inquiries and to make recommendations regarding their continuance or otherwise during 1950-51.

(*i*) *Indigenous drugs inquiry under Bt.-Colonel Ram Nath Chopra at the Drug Research Laboratory, Jammu.*

The Chairman explained that the main object of the scheme was the survey of drugs available in Northern India. They have now taken up the question of establishing a Herbarium under the auspices of the Drug Research Institute, Srinagar. They had investigated about sixty plants containing essential oils and these had been divided into three groups, i.e. (*i*) those containing a higher percentage of essential oils ; (*ii*) those of which the essential-oil content was low but could be improved and (*iii*) those where the essential-oil content was negligible. The development of these plants would be useful in supplying the necessary essential oils for perfumery and soaps. They have established now a farm for the cultivation of locally grown plants and their substitutes where the plants having lower content of essential oils in (*ii*) above would be cultivated with a view to improve their essential-oil content. They also had a proposal to study the anti-biotic side of the work in the various medicinal plants in addition to the active principles. Colonel Chopra's original scheme was supplemented by another request costing about Rs. 725. The Committee agreed to the continuance of the scheme *in toto*. Colonel Chopra made a proposal in the supplementary request to raise the status of the Assistant Research Officer to that of the Research Officer in view of the valuable work done by him in this field of research. The scale of pay of the Assistant Research Officer was Rs. 275-25-500-E.B.-30-590 and the present incumbent was getting Rs. 325 in that scale. It was agreed to recommend that he should be appointed Research Officer in the scale Rs. 350-350-380-30-590-E.B.-30-770-40-850 and that he should start at the lowest of the scale. In making this recommendation it was pointed out by Colonel Chopra that the only expenditure incurred by him in these schemes was on staff. With regard to all other contingent expenditure, laboratory appliances and equipment, these were met from the Drug Research Laboratory which was thus indirectly a source of saving to the Indian Research Fund Association. As the whole grant was for the minimum staff required, it was not possible to effect any cuts. The Pharmacological Assistant was, however, on a lower grade, so saving will be effected.

(*ii*) *Indigenous drugs inquiry under Dr. J. C. Gupta at the School of Tropical Medicine, Calcutta.*

The work done under this inquiry was explained by Dr. Gupta. It was pointed out that as against Rs. 34,338 budgeted for the year 1949-50 he had suggested a budget of Rs. 30,690 only. The Committee noted the reduction but agreed that a further reduction of Rs. 1,000 may be made under chemicals, crude drugs, contingency, etc., bringing the total expenditure to Rs. 29,690.

(*iii*) *Inquiry on the action of snake venoms and their constituents on the bio-chemical activity of tissue-cells under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta.*

The Committee discussed this scheme and recommended the following :—

The proposal to appoint a Laboratory Assistant in the scheme should be deleted and the Assistant Research Officer should be appointed on Rs. 275 as against Rs. 300 paid to the present incumbent who was leaving. This will result in a saving of Rs. 1,020 under 'Salaries' and corresponding expenditure in dearness and house-rent allowances.

Item 2.—To consider proposals for new inquiries.

(*i*) *Inquiry to carry out the chemical investigation of poisonous plants in India under Dr. R. N. Chakrabarti at the School of Tropical Medicine, Calcutta.*

The Committee discussed the proposal and decided that the work is of the same type as is being carried out by the indigenous drugs inquiry under Dr. J. C. Gupta. Dr. Chakrabarti can work in collaboration with Dr. Gupta. The scheme was not recommended.

(*ii*) *Inquiry to carry out toxicity tests of synthetic micro-filaricidal compounds under Dr. J. C. Gupta at the School of Tropical Medicine, Calcutta.*

The Committee considered that the scheme proposed by Dr. J. C. Gupta could be worked by him with the grant he gets for indigenous drugs work. Dr. Gupta agreed to this.

Similar work was done by Dr. K. V. Venkatraman, Director, King Institute, Guindy, Madras and he had proposed that with a contingent grant of Rs. 500 he would be able to continue the same. This was agreed to.

Item 3.—To consider a proposal from Major G. S. Chopra, Deputy Assistant Director-General, Medical Store Depot, Madras, for writing a comprehensive review on the work done on drug addiction under the auspices of the Indian Research Fund Association.

It was pointed out that the World Health Organization were interested in this aspect of the question and this work was very useful from the public health point of view. It was necessary to bring the work done by the drug addiction inquiry financed by the Indian Research Fund Association up to date but in view of the financial stringency this may be deferred to the next year.

Item 4.—To consider the question of import of drugs into India.

Mr. Nabar explained the general position with regard to the importation of drugs from foreign countries. It was pointed out by him that the question of import of drugs was decided by the Chief Controller of Imports in consultation with a committee consisting of representatives of the Ministry of Health and the Ministry of Industries & Supply. Only essential drugs were allowed to be imported. Drugs which were manufactured in India in sufficient quantities and those which were non-essential drugs, including patent and proprietary medicines, were not allowed to be imported. As far as dollar areas are concerned, 15 items of their preparations were allowed to be imported. In regard to the soft currency areas also, an essential list of drugs was drawn up and only those drugs or preparations containing those drugs were allowed to be imported.

The Committee considered the question of permitting importation of liver extracts for parenteral use, in view of the rapidly increasing output of Indian manufactured liver extracts in such Centres as Bombay and Calcutta. It noted the fact that the Pharmacopœia of the United States (13th Revision) had laid down standards and methods for testing the quality of the liver extracts. No liver extracts are allowed to be sold or exported from the U.S.A. unless they have been *clinically tested* and the unitage marked on the labels under the control of the U.S.P. Anti-anæmia Preparations Advisory Board (copy of entry in the U.S. Pharmacopœia is given in the Appendix). This ensures the quality of liver extracts for parenteral use. The Committee, therefore, are of the opinion that these tested liver extracts with definite unitage marked on the label from the U.S.A. should be allowed to be imported as no product made in India can take their place at present.

The Committee regretted that the production of liver extracts in India has still not been put on a sound basis. The livers obtained in India may sometimes be of comparatively poor quality because there is no stringent control over the slaughter of animals in poor physique and matters are made still worse by lack of adequate arrangements for collection and storage of organs in different parts of India. Because of these circumstances, the Committee also recommends that certain amount of liver extracts of high concentration for oral administration, which can only be obtained from abroad at present, should also be allowed to be imported, according to the demands of clinicians and hæmatologists.

The following resolutions were passed by the Committee :—

(*i*) The Committee is of the opinion that the authorities responsible for controlling the import and distribution of essential drugs should take adequate action to ensure that the essential drugs are made available to the people.

(*ii*) In view of the recent progress made in anti-biotics research and of the possibility that there may be potent anti-biotic substances available from Indian fungi, lichens and higher plants, the Committee suggests that the attention of workers should be drawn to the desirability of research in these fields.

APPENDIX.

Pharmacopœia of the United States, Thirteenth Revision.

Liver Injection—Liver Extract for Parenteral use.

Liver Injection is a sterile solution in water for Injection of that soluble thermostable fraction of mamalian liver which increases the number of red blood corpuscles in the blood of persons affected with pernicious anæmia. The approximate anti-anæmia potency of Liver Injection upon intramuscular administration in pernicious anæmia is expressed in U.S.P. units (Injectable.) Liver Injection contains not more than 15 U.S.P. Units (Injectable) in each c.c. Liver Injection conforms to all other requirements outlined under antianæmia Preparations, page 617. It meets the requirements of the Sterility Test for Liquids, page 689.

Sterilize Liver Injection preferably by Process F. See Sterilization Processes, page 692.

Liver Injection contains not more than 0·5 per cent cresol or of phenol if either is used as a bacteriostatic agent.

Liver Injection also conforms to the other requirements under Injections, page 664, except that at times it may show signs of as light turbidity or precipitate.

Packaging and storage, labelling, and sizes outlined in the U.S.P. are omitted.

Average Daily Dose—Intramuscular, i. U.S.P. Unit.

Pages referred to are listed in the United States Pharmacopœia, Thirteenth Revision.

Page 617 U.S.P. Thirteenth Revision states that anti-pernicious anæmia preparations must meet the following standards :

Anti-anæmia Preparations.

To meet the specifications of this Pharmacopœia, Liver, Stomach, or other preparations intended for the treatment of addisonian pernicious anæmia are to be approved by the U.S.P. Anti-anæmia Preparations Advisory Board.

A. Applications for approval shall be submitted to the Chairman of the Board by manufacturers on special forms, seven copies being required.

B. The application shall include :

(1) Statement of the species of animal liver or stomach used.

(2) Statement of the conditions under which the raw material, is obtained, handled, and stored prior to manufacture.

(3) Description of the facilities used in collecting, transporting, storing, manufacturing, and packing the article.

(4) Description of the methods and the controls used for the manufacture, processing, and the packaging of the drug. Description and composition of the final product with a statement of the total solids and amount of the preparation in final form derived from

100 gm. of original organ. If a product contains material derived from more than one source, such data should be given with respect to each components. The pH of products for parenteral use.

(5) The description of the controls employed in assuring uniformity in strength, quality, and purity of the drug, and of maintaining the identity of each lot.

(6) Clinical data from the treatment of cases of Addisonian pernicious anæmia in a specified manner with the product in question. Seven copies of the data shall be presented to the Board on special forms supplied by the Board.

(7) All labelling to be used upon the finished product, seven copies being required.

C. (1) If the data submitted satisfy the Board that the product manufactured as described and having the potency indicated by the clinical data is of a suitable strength, quality, and purity for the treatment of Addisonian pernicious anæmia, the Board will inform the applicant of the approval of the product and will assign a potency for it.

(2) The potency of preparations as defined by the Board shall be stated on the label as follows :

> 1 (c.c., gm., capsule, etc.) of material prepared by the method employed in producing the contents of this (bottle, vial, package) constitute(s) (no.) U.S.P. units (oral or injectable).

or alternatively as follows :

> ......(no.) (gm., or c.c.) (capsules, teaspoons, etc.) of material prepared by the method employed in producing the contents of this (bottle, vial, package) constitute(s) 1 U.S.P. unit (oral or injectable). (Unless the small size of individual containers (e.g. 1 or 2 c.c.) requires abbreviation for adequate legibility).

(3) The labelling shall bear a statement of the average dose of the product. The dose stated shall be a quantity which provides administration at the rate of not less than 1 U.S.P. unit a day whether given daily or at longer intervals.

D. A U.S.P. unit is that amount of an otherwise acceptable product which produces, when administered daily, clinical and hematopoietic responses in Addisonian pernicious anæmia that are considered by the Board to be satisfactory.

E. Approved liver, stomach, or other preparations relabelled or repackaged by a person other than the original manufacturer or packer are required to have the approval of the Board.

MISCELLANEOUS RESEARCHES.

1. Inquiry on filterable viruses under the Director, King Institute Guindy, Madras.

A. Influenza virus.

Diagnosis of influenza.—The determination of the virus inhibition titre of sera received in the routine course for serological tests was continued. The results are shown in Table I:—

Table I.

Agglutination inhibition titre of human sera with influenza viruses A and B.

Antigen.	Below 64	64	128	256	512	1,024 and above.	Total
A ..	30	75	156	105	41	10	417
B ..	22	34	84	96	44	13	293

Effect of bacterial enzymes on virus receptors.—The effect of enzyme preparations from different bacteria on their action of influenza virus by susceptible tissue-cells was studied. The technique reported last year was followed.

The following organisms were taken up for study, viz. (*i*) *C. diphtheriæ ;* (*ii*) *B. subtlis ;* (*iii*) *Proteus vulgaris ;* (*iv*) *B. paratyphoid A ;* (*v*) *B. paratyphoid B ;* (*vi*) *B. paratyphoid C ; and* (*vii*) *Pneumococcus.* The results are incorporated in Table II :—

Table II.

Enzyme preparation from :	Titration of the samples of fluid from the allantoic cavity at the end of :				
	30 min.	60 min.	120 min.	180 min.	240 min.
C. diphtheriæ ..	1 : 4	1 : 32	1 : 64	1 : 128	1 : 256
B. subtlis ..	1 : 8	1 : 32	1 : 64	1 : 256	1 : 256
Proteus vulgaris ..	1 : 128	1 : 192	1 : 128	1 : 256	1 : 256
B. paratyphoid A	1 : 4	1 : 16	1 : 64	1 : 128	1 : 128
B. paratyphoid B	1 : 4	1 : 32	1 : 64	1 : 128	1 : 128
B. paratyphoid C	1 : 8	1 : 32	1 : 128	1 : 128	1 : 256
Pneumococcus ..	1 : 128	1 : 128	1 : 192	1 : 192	1 : 192
Control ..	1 : 4	1 : 32	1 : 64	1 : 256	1 : 256

It is seen that of the different bacterial enzymes taken up for study, besides *V. choleræ,* only *Proteus vulgaris* and *Pneumococcus* prevented the absorption of influenza 'A' virus by susceptible cells. The rest had no appreciable effect.

Studies on the hæmagglutination of influenza 'A' virus by fowl red cells.—It was reported by Lannin and Beard (1948) that fresh egg-white was very effective in inhibiting the hæmagglutination by heated swine influenza virus.

Attempts were, therefore, made to study whether fresh egg-white had a similar effect on the PR 8 strain of influenza 'A' virus.

The virus used in the present work was from a pool of allantoic fluid from eggs infected with the agent and harvested in the usual manner. It had been stored at +4°C.

The diluent for carrying out the hæmagglutination test throughout was 0·85 per cent sodium-chloride solution.

The egg-white was separated from the yolk from several fresh eggs and filtered twice through a single layer of gauze. Further homogenization was accomplished by repeated pipetting. All fluids were stored at +4°C.

To each of a series of tubes containing 0·25 c.c. of increasing two-fold dilution of egg-white (starting with a 1 : 10 dilution) 0·25 c.c. of a virus suspension containing 8 hæmagglutinating units was added and the contents allowed to mix by shaking. They were then left at room temperature for 30 minutes. Then 0·5 c.c. of 1·5 per cent suspension of chicken erythrocytes was added to all the tubes and they were incubated at 22°C. Results were read after 75 minutes.

A parallel titration of the virus dilution used in the inhibition test was made in every experiment to insure that the right amount of virus had been used.

The experiment cited above was carried out with (*i*) active untreated virus and (*ii*) virus previously heated at 56°C. for 30 minutes.

The results are given in Table III :—

TABLE III.

Effect on egg-white on the hæmagglutinating activity of PR 8 strain of influenza A virus.

Reagents.	Reciprocal of final dilution of the incubated mixture.						
	10	20	40	80	160	320	640
Egg-white control (no virus)	−	−	−	−	−	−	−
Virus control (no egg-white) ..	+	+	+	+	±	−	−
Untreated active virus ..	±	+	+	+	+	+	+
Heated virus ..	−	−	−	±	+	+	+

It is seen from Table III that egg-white causes a significant inhibition of hæmagglutination in the case of heated influenza 'A' virus.

In view of the inhibitory activity of the egg-white on the hæmagglutination attempts were made to isolate the different proteins from it in order to identify that fraction of the egg-white which was responsible for this inhibitory activity.

The following two fractions of the proteins from egg-white were prepared, viz. ovomucin and egg albumin. Ovomucin was prepared following mainly the procedure described by Young (1937) and egg albumin was prepared according to Cole (1933).

Inhibition tests were carried out with the two preparations and their results are given in Table IV. It will be seen that of the two proteins taken up for study, ovomucin has greater inhibitory power to inhibit hæmagglutination by heated PR 8 'A' virus of influenza.

TABLE IV.

Strain.	Treatment.	Inhibition titre of	
		Ovomucin.	Egg albumin
PR 8 strain of influenza 'A' virus	Unheated active virus	<10	20
	Heated virus 30 min. 56°C.	80	40

B. POLIOMYELITIS.

The two strains of poliomyelitis virus are being maintained in monkeys. The local strain 'Bari' is in its 6th passage and the Car Nicobar Island strain 'Nicholas' is also in its 6th passage.

C. VACCINIA.

The two strains of vaccinia virus cultivated on the chorioallantoic membrane of the developing chick are being maintained. One strain is in its 156th passage and the other in its 58th passage.

V. INDIAN JOURNAL OF MEDICAL RESEARCH AND INDIAN MEDICAL RESEARCH MEMOIRS.

1. (*a*) *The Indian Journal of Medical Research, Volume XXXVI*, 1948.—The usual four numbers of the Journal were published and these appeared in January, April, July and October, respectively. The average size of each number was 105 pages of printed and tabular matter, with two plates, representing an increase of 20 pages per number as compared with Volume XXXV.

(*b*) *The Memoirs.*—No *Memoir* was published with Volume XXXVI.

(*c*) *Cost.*—The net cost of publication of Volume XXXVI (four numbers) was Rs. 8,567.

2. (*a*) *The Indian Journal of Medical Research, Volume XXXVII*, 1949.—Two numbers, i.e. the January 1949 and April 1949, have so far appeared. The July number is not expected to be out before end of December. October number which is already in the press may not be out before February 1950. January issue contained 111 pages and 2 plates and April issue 136 pages, while July and October issues will have approximately 120 pages and 130 pages with 5 plates, respectively. Volume XXXVII will, therefore, be about 88 pages larger than Volume XXXVI. There is now no dearth of suitable material and it is hoped we will be able to attain to our normal size of 160 pages per issue next year.

(*b*) *The Memoirs.*—*Memoir* No. 35 ('Exact Significance of Difference in Responses under Two Treatments' by Satya Swaroop) has been published with this Volume.

3. *Distribution* (1949).—

Indian subscribers	72	= 187
Foreign subscribers	115	
Exchange list	191	= 312
Free list	121	
Voucher copies to advertisers, regn., make-up, police, specimen, propaganda, etc.		20
Balance left with publishers for sale		181
Total printed		700

4. *Distribution lists.*—The existing lists were last revised in July 1938. The revision of these lists is in hand and the Editor's suggestions have already been forwarded to the Secretary, Indian Research Fund Association.

5. *Number of copies printed.*—Only 700 copies of the *Journal* have been printed since 1940 instead of the previous 900. This number is considered sufficient to meet all current demands. Vols. I-VIII of the *Journal* are out of print.

6. *Cost of printing the Journal.*—The following statement shows the cost of production during the last five years :—

Cost of printing.

Number	XXXII 1944	XXXIII 1945	XXXIV-XXXV 1946-47	Number	XXXV-XXXVI 1947-48	XXXVI-XXXVII 1948-49
	Rs.	Rs.	Rs.		Rs.	Rs.
May ..	2,116	4,037	4,086	April ..	2,274	3,490
				July ..	3,731	2,894
October ..	2,752	2,777	2,778	October ..	3,065	3,310
			Jan. 1947 1,447	January ..	1,907	2,729
Totals ..	4,868	6,814	8,311	Totals ..	10,977	12,423
Average pages	* 126	* 160	† 122	Average pages	‡ 93	‡ 115

* Two numbers only. † Three numbers (1946-47). ‡ Four numbers (1947-49)

Recoveries.

	Rs.	Rs.	Rs.		Rs.	Rs.
May ..	1,293	1,842	1,204	April ..	705	964
				July ..	3,102	964
October ..	*Nil*	1,580	1,204	October ..	3,102	964
			Jan. 1947 *Nil*	January ..	*Nil*	964
Totals ..	1,293	3,422	2,408	Totals ..	6,909	3,856

7. *Net cost of Journal.*—The following statement shows the net cost of the production of the *Journal* during the last five years :—

Journal.

Number.	1944	1945	1946-47	Number.	1947-48	1948-49
	Rs.	Rs.	Rs.		Rs.	Rs.
May ..	823	2,194	2,882	April ..	1,569	2,526
				July ..	629	1,930
October ..	2,752	1,197	1,574	October ..	−37	2,346
			Jan. 1947 1,447	January ..	1,907	1,765
Totals ..	3,575	3,391	5,903	Totals ..	4,068	8,567

The net cost during 1948-49 after deduction of receipts was Rs. 8,567, approximately double of what it was last year. The average number of pages per issue has increased this year by about 20 per cent and more tabular matter has been printed. This together with delay in receipt of outstanding subscriptions and advertisement charges is responsible for the apparent increase in the net cost of production. The Publishers' bills for 1948-49 were submitted in time to make payments within the financial year.

8. *Financial position for the year* 1949-50.—The budget for the year provided for Rs. 14,000 to meet the cost of four quarterly numbers of the *Journal* and Rs. 2,000 for any *Memoir* which may be published. Estimated expenditure during the year is as follows :—

Cost of printing.—

Journal.—

	Rs.
Volume XXXVI, Nos. 2, 3 and 4 (April, July and October 1949) @ Rs. 2,500/- each Volume XXXVII, No. 1 (January 1950) @ Rs. 2,500	10,000
Memoir No. 35	2,900
Total ..	12,900

Saving anticipated Rs. 3,100.

9. *Editorial policy.*—The editorial policy of the *Journal* was revised in 1945 on the recommendations of the Scientific Advisory Board and the Editorial Board was enlarged to include 25 referees. A note for the guidance of members of the Board was also prepared by the Editor and circulated to all concerned. The services of the members of the Board have been fully utilized in accordance with the policy adopted. The number of members on the Editorial Board having fallen to 15, due to many officers having retired from service, the question of revision of the list was taken up during the year and it has now been brought to a strength of 30.

All the papers published in Vol. XXXVII were submitted by workers in India or were the results of investigations carried out in India. The lines of special research activity are reflected in the nature of papers received. Of the 47 articles which have been published or accepted for publication, 22 were on nutrition, 11 on bacteriology, 3 on biochemistry, 1 on snake venoms, 1 on pharmacology, 3 on haematology and 6 on other subjects.

The size of the *Journal* was reduced during the war owing to reduction in the number of papers received for publication, but it is hoped that with the revival of research in India enough material will now be forthcoming to bring the *Journal* in its pre-war size as is now indicated by the gradual increase in the number of papers received.

VI. INDIAN JOURNAL OF MALARIOLOGY.

The Indian Journal of Malariology, Volume II, Nos. 1, 2, 3 *and* 4, 1948.—The combined March and June 1948 issue consisting of 108 pages and the September 1948 and December 1948 numbers comprising 110 and 109 pages, respectively, of printed and tabular matter, were published during the year under report.

All papers incorporated in the above volume were submitted by workers in India or were the results of investigations carried out in India.

The Indian Journal of Malariology, Volume III, 1949.—The March 1949 number is in the Press and is expected to be ready for distribution shortly.

For reasons of delay in the printing of the *Journal* by the publishers and partly due to the number of articles received for publication being small, it was considered advisable to combine the June and September numbers. This is also in the Press and is likely to be ready for distribution in November or December, 1949.

Manuscripts for the December 1949 issue are being sent to the Press.

Distribution.—The exchange and free mailing lists were revised and names of certain institutions and individuals were deleted and some added. The number of foreign subscribers has shown an increase. The following Table gives the present distribution list :—

Indian subsrribers	94	= 160
Foreign subscribers ..	66	
Exchange list	80	= 178
Free list	98	
Voucher copies to advertisers, specimens, propaganda, etc.		20
At the Malaria Institute for distribution		50
Balance left with the publishers for sale		192
Total printed ..		600

Since the cost of the printing has not been reduced, the annual subscription of the *Journal* will remain the same, i.e. Rs. 12/- per volume consisting of four numbers and single copy at Rs. 5/-.

VII. PAPERS PUBLISHED ON MEDICAL RESEARCH CARRIED OUT UNDER THE AUSPICES OF THE INDIAN RESEARCH FUND ASSOCIATION DURING THE YEAR 1949.

A. *Indian Journal of Medical Research* and *Indian Medical Research Memoirs.*

1. BAHL, A. N., SADANA, J. C., and AHMAD, BASHIR. Carotenoid Pigments of different Cross-breeds of Cultivated and Wild Varieties of Tomatoes and the Effect of further Inter-varietal Crossing on the Carotenoid Content (Vol. 37, No. 2).
2. BOSE, S. M., and SUBRAHMANYAN, V. Examination of Indian Shark-liver Oils for Vitamin A and some Analytical Characteristics : Relationship between the Extinction Coefficient of Carr-Price Reaction at its Absorption Maximum and International Units (Vol. 37, No. 1).
3. BOSE, S. M., and SUBRAHMANYAN, V. Effect of some Factors on the Protection of Vitamin A in Shark-liver Oil by Anti-oxidants (Vol. 37, No. 1).
4. CHAKRAVARTY, N. K., SEN GUPTA, P. C., BOSE, J. P., and DE, U. N. Biochemical Investigations in Kala-azar. Part I. The Hepatic Function in Kala-azar (Vol. 37, No. 2).
5. CHAKRAVARTY, N. K., SEN GUPTA, P. C., BOSE, J. P., and DE, U. N. Biochemical Investigations in Kala-azar. Parts II and III (Vol. 37, No. 4).
6. CHATTERJEE, A. K. Studies on Snake Venoms. Part I. The Isolation of an Inhibitor of the Cytochrome-cytochrome Oxidase System from Cobra Venom and Study of the Effects of pH, Temperature and Ultra-violet Rays on its Stability (Vol. 37, No. 2).
7. CHOPRA, G. S., and CHOPRA, R. N. Opium Addiction in Assam and Attempts made to Eradicate it (Vol. 37, No. 4).
8. DAS GUPTA, C. R., and CHATTERJEA, J. B. Folic Acid in the Treatment of Macrocytic Anæmia in Pregnancy (Vol. 37, No. 4).
9. DE, H. N. Copper and Manganese Metabolism with Typical Indian Dietaries and Assessment of their Requirement for Indian Adult (Vol. 37, No. 3).
10. DE, H. N., and BANERJEE, S. N. Mutual Influence of Nicotinic Acid and Riboflavin in Metabolism (Vol. 37, No. 3).
11. DE, H. N., and BARAI, S. C. Study of the Mechanism of Biosynthesis of Ascorbic Acid during Germination (Vol. 37, No. 1).
12. DE, H. N., and BASU, K. P. Mutual Influence of Minerals in Metabolism (Vol. 37, No. 2).
13. DESIKACHAR, H. S. R., and SUBRAHMANYAN, V. Infant Feeding Experiments with Soya-bean Milk (Vol. 37, No. 1).
14. DESIKACHAR, H. S. R., and SUBRAHMANYAN, V. The Level of Protein Intake and the Quality of Protein on Calcium and Phosphorus Absorption (Vol. 37, No. 1).
15. DEVADAS, P. R. Analyses of South Indian Food Preparations (Vol. 37, No. 1).

16. DAYAGUDE, R. G., and BANKER, D. D. Treatment of Typhoid Fever with Bacteriophage : A Preliminary Report (Vol. 37, No. 3).
17. DHAYAGUDE, R. G., and PURANDARE, N. M. Studies on Anærobic Wound Infection (Vol. 37, No. 3).
18. KOTHARI, B. V., and BHENDE, Y. M. Nutritional Megaloblastic Anæmia : So-called Pernicious Anæmia of Pregnancy : A Preliminary Report (Vol. 37, No. 3).
19. KRISHNA MURTI, and SUBRAHMANYAN, V. The Effect of Certain Supplements on the Growth of Young Rats on Poor South Indian Rice Diet (Vol. 37, No. 1).
20. KUPPUSWAMY, S., GIRI, K. V., and SUBRAHMANYAN, V. Supplementary value of Oil-seed Cakes to South Indian Diet (Vol. 37, No. 1).
21. PANDIT, C. G., and VENKATRAMAN, K. V. On Reactions following Re-vaccinations with Vaccinia Virus Preparations, with Special Rerference to the 'Reaction of Immunity' (Vol. 37, No. 3).
22. PATWARDHAN, V. N., MUKUNDAN, R., RAMA SASTRI, B. V., and TULPULE, P. G. Studies in Protein Metabolism. The Influence of Dietary Protein on the Urinary Nitrogen Excretion (Vol. 37, No. 3).
23. RAMALINGASWAMI, V., and MENON, P. S. Folic Acid in Nutritional Macrocytic Anæmia (Vol. 37, No. 4).
24. RAMALINGASWAMI, V., and PATWARDHAN, V. N. Diet and Health of the South Indian Plantation Labour (Vol. 37, No. 1).
25. RAMAMURTI, K., and BANERJEE, B. N. Studies of Indian Edible Oils. Sesame Oil (Vol. 37, No. 4).
26. RANADIVE, KAMAL J. Comparative Response of Mammary Glands to early Gonadectomy (Vol. 37, No. 4).
27. RANADIVE, KAMAL J. Effect of Foster-nursing on Maturation of Ovaries (Vol. 37, No. 4).
28. RANGANATHAN, S., and PATWARDHAN, V. N. Alkaline Phosphatase in Erythrocytes (Vol. 37, No. 2).
29. SADANA, J. C., and AHMAD, BASHIR. Metabolism of the Carotenoid Pigments of the Mango during the Development of the Fruit (Vol. 37, No. 2).
30. SADANA, J. C., and AHMAD, BASHIR. The Stability of added Carotene in Vanaspati under different Conditions of Storage (Vol. 37, No. 2).
31. SATYA SWAROOP. Exact Significance of Difference in Responses under Two Treaments (*Ind. Med. Res. Memoir* No. 35).
32. SUBRAHMANYAN, V., and SUR, B. K. Supplementary Value of Lucerne to the Poor South Indian Rice Diet (Vol. 37, No. 3).
33. THEOPHILUS, F., and ARULANANTHAM, R. Analyses of some Edible Green Leaves in South India (Vol. 37, No. 1).

B. *Indian Journal of Malariology.*

1. DAKSHINAMURTY, SONTI, and SHARMA, M. I. D. A Micro-hygrometer (Vol. 3, Nos. 2 and 3).
2. JASWANT SINGH, and DALIP SINGH. Control of Rural Malaria with DDT indoor residual spraying in Delhi Province during the year 1948 (Vol. 3, Nos. 2 and 3).
3. JASWANT SINGH, and KARIAPA, C. B. Malaria Control in Coorg (Vol. 3, Nos. 2 and 3).
4. JASWANT SINGH, RAY, A. P., and NAIR, C. P. Transmission Experiments with *P. knowlesi* (Vol. 3, Nos. 2 and 3).
5. MULLIGAN, H. W., SOMMERVILLE, T., and LLOYD, O. C. Observations on the Infectivity of Tissues of *Macaca mulatta* during the Incubation period following Exposure to Infection with Sporozoites of *Plasmodium cynomolgi* (Vol. 3, Nos. 2 and 3).
6. RAGHAVAN, N. G. S. A New Method of Diagnosis of Kala-azar (Vol. 3, Nos. 2 and 3).
7. RAGHAVAN, N. G. S., and KRISHNAN, K. S. Some Observation on the Prevalence of Malaria and Filariasis in Sri Harikotta Island, Nellore, Madras Presidency (Vol. 3, No. 1).
8. RAGHAVAN, N. G. S., and KRISHNAN, K. S. A Note on Experimental Infections of *Mf. malayi* Brug. in *C. fatigans* and *A. stephensi* (type) (Vol. 3, Nos. 2 and 3).
9. RAGHAVAN, N. G. S., and MISRA, B. G. A Preliminary Note on Experimental Sauropsidal Malarial and Filarial Infections in some *C. fatigans* Weidemann, 1828 (Vol. 3, Nos. 2 and 3).
10. RAGHAVAN, N. S. G., and SATYA PRASKAH. A Preliminary Note on Napier and Chopra Tests carried out in 'Reconstitute Sera' of Kala-azar Cases (Vol. 3, Nos. 2 and 3).
11. RAMAKRISHNAN, S. P., KRISHNAN, K. S., and RAMAKRISHNA, V. Report on a Pilot Scheme for Malarial Control in the Betelnut-growing areas in Puthur Taluk, South Kanara District, Madras Province, 1947-48 (Vol. 2, No. 4).

C. *List of papers published by workers under the Indian Research Fund Association in Journals other than the 'Indian Journal of Medical Research' and the 'Indian Journal of Malariology'.*

1. BHATE, D. S., PANSE, T. B., and VENKATRAMAN, K. 3 : 3^1-di-hydroxy-azobenzene-4 : 4^1-di-carboxylic acid and 4-amino-salicylic acid (*Proc. Ind. Acad. Sci.*, Vol. 1949).
2. DAS GUPTA, C. R., and CHATTERJI, J. B. Magnkaryocyte in Peripheral Blood (*Ind. Jour. Med. Assoc.*, Vol. 18, No. 6).
3. DHARMENDRA. Some Observations on Sulphetrone Treatment of Leprosy (All-India Leprosy Workers' Conference, Calcutta, 1948).
4. DHARMENDRA. A Note on the Histopathhlogy of Leprosy (All-India Leprosy Workers' Conference, Calcutta, 1948).

5. DHARMENDRA. Some Comments on the Classification of Leprosy recommended by the Havana Congress (All-India Leprosy Workers' Conference, Calcutta, 1948).
6. DHARMENDRA. Leprosy Cases seen in the Indian Army (Official History of the World II War, 1949).
7. DHARMENDRA. Diet and Susceptibility to Leprosy (National Nutritional Association of India, Calcutta, 1949).
8. GORE, T. S., PANSE, T. B., and VENKATRAMAN, K. The Constitution of Citrinin (*Jour. Amer. Chem. Soc.*, Vol. 1949).
9. GORE, T. S., PANSE, T. B., and VENKATRAMAN, K. Critinin. Part I (*Proc. Ind. Acad. Sci.*, Vol. 1949).
10. PATWARDHAN, V. N. Nutritional Disease in India (*Antiseptic* June 1949).
11. SARMA, P. S., MENON, P. S., and VENKATACHALAM, P. S. Acetylation in the Laboratory Diagnosis of 'Burning-Feet Syndrome' (Pantothenic-acid Deficiency) (*Current Science*, September 1949).
12. BANERJEE, S., GHOSH, N. C., and BHATTACHARJEE, G. A Chemical method of Estimation of Nicotinic Acid in Urine in the Presence of Sugar (*Jour. Biol. Chem.*, Vol. 172, 1948).
13. BANERJEE, S., and GHOSH, N. C. Effect of Nicominide on Blood-Sugar Acetone Bodies of Diabetic and Normal Subjects (*Jour. Biol. Chem.*, Vol. 177 1949).
14. CHAUDHURI, S. N., and RAY, J. C. Nature and Specificity of Urinary Proteose. Part I. Clinical Trials of Auto-proteose in Allergic Cases (*Ann. Biochem. Exper. Med.*, Vol. 9, 1949).
15. CHAUDHURI, S. N., and RAY, J. C. Nature and Specificity of Urinary Proteose. Part II. Demonstration of Antibodies against Urinary Proteose, using it as an Antigen (*Ann. Biochem. Exper. Med.*, Vol. 9, 1949).
16. CHAUDHURI, S. N., and RAY, J. C. Nature and Specificity of Urinary Proteose. Part III. Study on the Relation of Urinary Proteose with the Bacteria suspected to be the Primary Factor for the Condition (*Ann. Biochem. Exper. Med.*, Vol. 9, 1949).
17. Nature and Specificity of Urinary Proteose. Part IV. Study of Anaphylaxis in Rabbit against Urinary Proteose (*Ann. Biochem. Exper. Med.*).
18. Study on the Biochemistry of Allergic Blood. Part I. Study on Sodium, Potassium, Chlorides and Protein Rations (*Ann. Biochem. Exper. Med.*).

VIII. PROGRAMME OF RESEARCHES AND MISCELLANEOUS GRANTS RECOMMENDED FOR THE YEAR 1950-51.

The following inquiries and budget allotments were recommended to the Governing Body by the Scientific Advisory Board at its meetings held on the 28th and 29th November, 1949.

INQUIRIES.

Cholera.

Continuation inquiries:—

		Budget allotments. Rs.
1.	Inquiry on cholera under the Director, Central Research Institute, Kasauli	18,506
2.	Inquiry on cholera under the Director, King Institute, Guindy, Madras	94,000
3.	Inquiry on cholera under Dr. S. Ghosal at the School of Tropical Medicine, Calcutta	13,915
4.	Inquiry into the endemicity of cholera in India under Dr. Satya Swaroop at the All-India Institute of Hygiene & Public Health, Calcutta	7,980
5.	Inquiry on cholera under Dr. K. V. Krishnan at the All-India Institute of Hygiene & Public Health, Calcutta ..	13,605

Malaria.

Continuation inquiries:—

6.	Insecticide and mosquito repellent inquiry under the Director, Malaria Institute of India, Delhi	44,483
7.	Inquiry on malaria under Dr. R. N. Chaudhuri at the School of Tropical Medicine, Calcutta	10,505
8.	Inquiry on the chemotherapy of malaria under Dr. K. Ganapathi at the Haffkine Institute, Bombay ..	20,773

Nutrition.

Continuation inquiries:—

9.	Nutrition Research Laboratories, Coonoor	2,02,255
10.	Nutrition research unit under Dr. B. C. Guha, at the University College of Science & Technology, Calcutta ..	26,918
11.	Inquiry on the metabolism of vitamin A under Dr. K. Rajagopal at the All-India Institute of Hygiene & Public Health, Calcutta	7,025
12.	Inquiry into the physiological effects on dietary protein under Dr. M. Damodaran at the National Chemical Laboratory, Poona	13,264
13.	Inquiry into the nutrition value of diets prescribed for hospital patients in India under Dr. K. Mitra, Adviser on Nutrition, Directorate-General of Health Services, New Delhi	2,400

INQUIRIES.

		Budget allotments. Rs.
14.	Inquiry on the studies on the bio-synthesis of nicotinic acid under Dr. S. Banerji at the Presidency College, Calcutta ..	11,382
15.	Nutrition research unit under Dr. S. S. De at the Indian Institute of Science, Bangalore	9,430
16.	Inquiry into the effects of malnutrition on the physique and more particularly on the blood picture including hæmoglobin and plasma-protein concentration as also the treatment thereof with high protein diets, protein hydrolysates, etc., under Prof. (Miss) S. Chaudhuri and Dr. K. Mitra at the Lady Hardinge Medical College, New Delhi ..	6,004
17.	Nutrition research unit under Dr. R. G. Chitre at the Seth G. S. Medical College, Bombay	11,000
	New inquiries:—	
18.	Microbiological investigations in the B-group of vitamins under Dr. A. Sreenivasan at the Department of Chemical Technology, University of Bombay, Bombay	6,030
19.	Inquiry on the enzymic role of inositol under Dr. P. S. Sarma at the University Biochemical Laboratory, University of Madras, Madras	3,880
20.	Inquiry to study the digestion, evacuation time and gastric response to various Indian food preparations under Dr. J. D. Pathak at the Topiwala National Medical College, Bombay	2,050
21.	Inquiry on the nutritive value of Indian dietary productions under Dr. K. P. Basu at the Indian Dairy Research Institute, Bangalore	6,264
22.	Inquiry to assess the value of supplementing rice diet with de-fatted groundnut cake flour fed to children under Dr. S. B. Lal, Officer-in-Charge, Nutrition Scheme, Bihar ..	2,960
	Leprosy.	
	Continuation inquiries:—	
23.	Leprosy inquiry under Dr. Dharmendra at the School of Tropical Medicine, Calcutta	19,890
24.	Study of histopathology of leprosy under Dr. R. G. Cochrane at the Lady Willingdon Leprosy Sanatorium, Chingleput ..	20,350
	Plague.	
	Continuation inquiries:—	
25.	Plague researches under the Director, Haffkine Institute, Bombay	26,450

Clincial research.

	INQUIRIES.	Budget allotments. Rs.
26.	Clinical research unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay	16,950
27.	Neuropathological unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay	24,950
28.	Inquiry on the role of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay	10,825
29.	Inquiry on the clinical and biochemical investigations on certain aspects of infantile hepatic cirrhosis under Drs. V. Subrahmanyan and V. R. Naidu at the Central Food Technological Research Institute and Sri Krishnarajendra Hospital, Mysore	11,450
30.	Inquiry on typhus in Bengal under Dr. K. V. Krishnan at the All-India Institute of Hygiene & Public Health, Calcutta	20,315
31.	Inquiry on the immunological properties of lens and uveal proteins under Dr. S. N. Cooper at the Seth G. S. Medical College, Bombay	8,600
32.	Inquiry on the chromatophorotropic hormone of the pituitary gland and its employment in the treatment of pigmentary disorders of the skin under Dr. B. Mukerji at the Central Drugs Laboratory, Calcutta	17,950
33.	Inquiry into the state of refraction of Indian children of different communities under Dr. G. S. Pendse at the Talegaon General Hospital, Poona	2,920
34.	Study of *Monila* infection of the skin and adjacent mucous membranes under Dr. L. M. Ghosh at the School of Tropical Medicine, Calcutta	4,760
35.	Inquiry on the problem of typing typhoid bacilli by means of Vi-bacteriophage and treatment of typhoid fever with type specific Vi-'phage under Dr. R. G. Dhayagude and Dr. D. D. Banker at the Seth G. S. Medical College, Bombay	3,956
36.	Inquiry on the circulatory changes in severe infection under Dr. R. N. Chaudhuri at the School of Tropical Medicine, Calcutta	8,643
37.	Hæmatological unit under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta	17,112
38.	Hæmatological unit under Dr. J. C. Patel and Dr. Y. M. Bhende at the Seth G. S. Medical College, Bombay ..	10,860
39.	Polio research unit under Dr. P. V. Gharpure at the Grant Medical College, Bombay	27,180
New inquiries :—		
40.	Studies on the role of fungi in pulmonary diseases under Dr. P. N. Wahi at the Medical College, Agra	3,000

	INQUIRIES.	Budget allotments. Rs.
41.	Investigation on the cultivation of *Entamœba histolytica* in a bacteria-free medium under Dr. J. C. Ray at the Indian Institute for Medical Research, Calcutta	8,395
42.	Inquiry on the relationship of pancreas to the content formation of lipocaic and ultimately the mode of the action in the control and regulation of fat metabolism under Dr. J. C. Sachdev at the Mahatma Gandhi Memorial Medical College, Indore	400
43.	Inquiry on epidemic dropsy under Dr. S. N. Sarkar at the Campbell Medical College, Calcutta	7,060
44.	Inquiry into seminal stains under Lieut.-Colonel S. D. S. Greval at the School of Tropical Medicine, Calcutta ..	3,000
	Maternity and child welfare.	
	Continuation inquiries :—	
45.	Inquiry into the morbidity of children between the ages of 1 and 5 years (pre-school) in the Singur Health Centre area under Dr. Muktha Sen at the All-India Institute of Hygiene & Public Health, Calcutta	8,710
	Filariasis.	
	Continuation inquiries :—	
46.	Filariasis inquiry under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta	8,670
47.	Inquiry on changing the physical properties of drugs by wetting agents under Dr. K. Venkatraman at the Department of Technology, University of Bombay, Bombay ..	9,480
48.	Filariasis control unit under the Director, Malaria Institute of India, Delhi	40,330
	Rabies.	
	New inquiries :—	
49.	Research on rabies under the Director, Central Reserch Institute, Kasauli	5,150
	Industrial health.	
	Continuation inquiries :—	
50.	Industrial health research unit under the Director, All-India Institute of Hygiene & Public Health, Calcutta ..	47,310
51.	Inquiry into the environmental conditions such as lighting, temperature, humidity, ventilation and dust in certain industries in and around Calcutta under Prof. K. Subramanyan at the All-India Institute of Hygiene & Public Health, Calcutta	7,160

INQUIRIES.

		Budget allotments. Rs.
52.	Inquiry into the problem of traffic accidents and accident prone personnel amongst bus and tram drivers in Calcutta under Mr. S. K. Bose at the University College of Science & Technology, Calcutta	1,800
53.	Inquiry into the prevalence of byssinosis in cotton textile industries in South India under Dr. T. S. Adisubramaniam, Madras Public Health Service, Madras	7,370

Pharmacology.

Continuation inquiries:—

54.	Indigenous drugs inquiry under Brevet-Colonel Sir Ram Nath Chopra at the Drug Research Laboratory, Jammu	10,895
55.	Indigenous drugs inquiry under Dr. J. C. Gupta at the School of Tropical Medicine, Calcutta	29,690
56.	Inquiry on the action of snake venoms and their constituents on the biochemical activity of tissue cells under Dr. B. N. Ghosh at the University College of Science & Technology, Calcutta	6,615

Miscellaneous researches.

Continuation inquiries:—

57.	Statistical inquiry into the numerical estimation of *coliform* organisms in water samples by the dilution method under Dr. Satya Swaroop at the All-India Institute of Hygiene & Public Health, Calcutta	2,630
58.	Filterable viruses inquiry	10,042

GRANTS.

(*i*)	Office of the Secretary, Indian Research Fund Association	1,13,553
(*ii*)	Staff employed in the office of the Accountant-General, Central Revenues, for I. R. F. A. work	18,553
(*iii*)	I. R. F. A. reports and publications	13,000
(*iv*)	Travelling allowance of the members of the Governing Body, Scientific Advisory Board and Advisory Committees of the Indian Research Fund Association	20,000
(*v*)	Cost of two professorships at the School of Tropical Medicine, Calcutta	32,182
(*vi*)	Contribution to the Imperial Institute of Entomology, London	1,350
(*vii*)	Contribution towards the Bureau of Hygiene & Tropical Diseases, London	2,025
(*viii*)	Research fellowships	1,25,000

INQUIRIES.

			Budget allotments. Rs.
(*ix*)	*Indian Journal of Medical Research* and *Indian Medical Research Memoirs* :—		
	(*a*) Printing and publication charges of the *Journal*	13,000	
	(*b*) Printing and publication charges of *Memoirs*	2,000	
	(*c*) Editor's Office	10,982	25,982
(*x*)	*Indian Journal of Malariology* :—		
	(*a*) Printing and publication charges of the *Journal*	10,000	
	(*b*) Editor's Office	3,510	13,510
(*xi*)	I.R.F.A. library at the Central Research Institute, Kasauli.		700
(*xii*)	I.R.F.A. Microfilm Service Units		
	(*i*) at the Central Research Institute, Kasauli		30,660
	(*ii*) at the Tata Memorial Hospital, Bombay		1,740
(*xiii*)	Pay (including special pay and allowances) and contributions towards leave salary and pension or contributory provident fund of officers of the Medical Research Department under the Association		75,000
(*xiv*)	Travelling allowance and contingent expenses for Medical Research Department officers employed under the Indian Research Fund Association		10,000
(*xv*)	Clerical assistance at the School of Tropical Medicine, Calcutta		9,230
(*xvi*)	Clerical assistance at the All-India Institute of Hygiene & Public Health, Calcutta		3,880
(*xvii*)	Travelling allowance for Indian Research Fund Association workers on transfer		3,000
(*xviii*)	Indian Research Fund Association contribution and interest towards the Contributory Provident Fund		22,000
(*xix*)	Miscellaneous unforeseen expenditure		60,000
(*xx*)	Imprest advance for inquiries		11,200